Defining the genetic and physiological basis of *Triticum sphaerococcum* Perc.

A thesis submitted in partial fulfilment of the requirements for the degree

of

Master of Science in Cellular and Molecular Biology

at the

University of Canterbury

Puthiyaparambil Chacko Josekutty

2008

DEDICATION

This thesis is dedicated to my beloved parents with due gratitude for their love, support and encouragement which shaped up my life

Also

To my supervisor, Late Dr. Sandra Jackson, School of Biological, Sciences who introduced me to this project

ABSTRACT

Triticum sphaerococcum (AABBDD, 2n = 6x = 42) is a land race of wheat known from the Indian subcontinent. It has several favourable characters including short and strong culms, hemispherical grains with a shallow crease (that may increase the yield of white flour), higher protein content compared to bread wheat (*T. aestivum*), and resistance to drought, and yellow rust caused by *Puccinia striiformis.* However, an unfavourable characteristic of *T. sphaerococcum* is its lower yield compared to bread wheat. Being a land race, the sphaerococcum wheat is poorly studied. This study was undertaken to increase knowledge of the physiology and genetics of this land race and determine if it may be possible to separate the favourable characters of *T. sphaerococcum* from its unfavourable characters.

Plant height in bread wheat is controlled by many genes. 'Reduced Height' (*Rht*) genes which differ in their response to externally applied gibberellic acid (GA_3) are responsible for the short stature of modern bread wheat varieties. Therefore, GA_3 was used to probe the relationship between the semidwarf sphaerococcum phenotype and the *Rht* gene. *T. sphaerococcum* variety Sp5 showed a unique *"seedling response"* to externally applied GA_3 when compared with *T. aestivum* varieties harbouring *Rht1*, *Rht2*, *Rht8*, *Rht12*, *Rht13* or *Rht18* alleles.

A mapping population of doubled haploids was generated through wide hybridisation of F1 (Sp5 x Otane) with *Zea mays*. A genome-wide scan of Sp5 and Otane (parents) using 348 microsatellite (SSR) markers showed that only 169 of these markers (49%) were polymorphic between the parents. A DArT profiling yielded 348 markers that were polymorphic between the parents. Microsatellite markers and DArT markers were used to create a genetic map. The mapping population was phenotyped and a quantitative trait loci (QTL) analysis was performed for component traits of the complex sphaerococcum trait including plant height, spike length, awn length, yield, grain shape and crease size. Results of the QTL analysis indicated that it may be difficult to separate the favourable characters of *T. sphaerococcum* from its unfavourable characters through mutation because the component traits of the complex sphaerococcum trait may be under pleiotropic control of the *Sp* gene.

The hypothesis that *T. sphaerococcum* originated through a mutation in *T. aestivum* was tested through induced mutation using gamma rays. Mutants from sphaerococcum-type to aestivum-type were isolated and phenotyped. Sphaerococcum-type mutants also were isolated and characterised from mutated aestivum-type wheat suggesting a possible origin of *T. sphaerococcum* through a mutation in *T. aestivum*.

ACKNOWLEDGEMENTS

I am indebted to my supervisors Dr Ross Bicknell, Crop & Food Research, Lincoln, Prof Paula Jameson, Head, School of Biological Sciences and late Dr Sandra Jackson, UC, for the mentoring, supervision, constant encouragement and consistent support without which I could not have completed this project. Special thanks to Dr Bicknell and Mr Steve Shorter, CFR, Lincoln who allowed me to use materials and data from their previous research to speed up this project. Mr Shorter also supervised the plant breeding aspects like doubled haploid production and evaluation of the mutants in the field and the glasshouse. Without the assistance of Steve's team, it would not have been possible to carryout the elaborate field trials of the mutants and related processing. The majority of the doubled haploids used in this study came from the work of Dr Maqbool Ahmad at Crop & Food Research Lincoln. In addition to my supervisors, Dr Kevin Sutton and Dr Nick Ashby of CFR, Lincoln supported me with administrative matters relating to this project. Dr David Leung, UC is thankfully acknowledged for allowing the use of his laboratory for some experiments and encouragement.

Mrs Sylvia Erasmuson, CFR, Lincoln had been an excellent mentor, lab mate and friend who helped me to master techniques in genomics. Without her support and cooperation, it would have been extremely difficult for successfully carrying out this research and I sincerely thank her and rest of the apomixis team at CFR, Lincoln for their contributions. Mrs Vijitha Meialagan and Dr Richard Pickering, CFR, Lincoln is gratefully acknowledged for useful discussions and assistance with doubled haploid production. Meenken Esther and Ruth Butler, Statisticians at Crop & Food Research are gratefully acknowledged for their help with statistical design and analysis. Assistance of Mr Neil Hines, glasshouse manager at CFR was critical to this project.

Mr Dave Condor had been a great help at the UC glasshouse. Mr. Mathew Walters helped with the photography and Mr Manfred Ingerfield helped me with microscopy and in the laboratory work at UC. Dr Andrew Catanach, CFR, Lincoln is acknowledged for useful discussions and teaching some laboratory methods in genomics and operation of genetic analyser. Saira Wilson, Sheree Davis, Sathyamoorthy Meialagan and the student community in general at the CFR are

acknowledged for various helps rendered. My lab mates at the Cell Biology lab, UC are thanked for familiarising me with the lab during my early days. Assistance by management staff at Canterprise, UC and College of Science and School of Biological Science, at the University are gratefully acknowledged for their support in various ways. I gratefully acknowledge the help received from technical staff in the plant breeding division and plant genetic technology group.

Ms Cathy Hargreaves, my supervisor at SCION, Rotorua, is thanked for constant encouragement and support. Mr Chaley Low, Dr Sathish Kumar, Dr Philip Wilcox and Dr Emily Telfer, SSCION, Rotorua are acknowledged for useful discussions and assistance offered to improve parts of the manuscript. I gratefully acknowledge the support and help from Mr Vinod Babu, Mr Tony Joseph and Ms Lai Fern, fellow students at UC. I thank Ruby, my beloved wife for her understanding, patience, constant support and assistance with the thesis preparation.

TABLE OF CONTENTS

LIST OF FIGURES

ABBREVIATIONS

AFLP: Amplified fragment length polymorphism

BAP: 6-Benzylaminopurine

BP: Before Present

CAPS: Cleaved amplified polymorphic sequence

CFR: Crop & Food Research, Lincoln, New Zealand

Cm: Centimetre

cM: Centimorgan

CYMMIT: *Centro Internationale de Mejoramiento de Maiz Y Trogo* (International Maize and Wheat Improvement Centre)

DArT: Diversity Arrays Technology

Df: Degrees of freedom

DH: Doubled haploid

DMSO: Dimethyl sulfoxide

DNA: Deoxyribonucleic acid

dNTP: Dinucleotide triphosphate

EMS: Ethyl Methane Sulphonate

EST: Expressed sequence tag

EtOH: Ethyl Alcohol

FAM: 6-carboxy-fluorescine

FAO: Food and Agriculture Organisation

FAOSTAT: Statistics published by FAO

FRST: Foundation for Science Research and Technology, New Zealand

GA: Gibberellic Acid

GA_3: Gibberellic Acid 3

h: Hour

HCl: Hydrochloric Acid

HEX: Hexachloro-6-carboxy-fluorescine

IBA: Indole-3-butyric Acid

IRRI: International Rice Research Institute, Manila, Philippines

ITS: Internal transcribed sequence DNA

KOH: Potassium Hydroxide

LSD: Least significant difference

M: Molar

MAS: Marker Assisted Selection

MI: Mitotic index

MS Medium: Murashige and Skoog (1962) Medium

NaOAc: Sodium Acetate

NRL: National Radiation Laboratory, Christchurch, New Zealand

Ot: *T. aestivum* cv. Otane

PCR: Polymerase chain reaction

PFD: Photon flux density

QTL: Quantitative trait loci

RAPD: Random amplified polymorphic DNA

RFLP: Restriction fragment length polymorphism

Rht: reduced height gene

ROX: 6-carboxy rhodamine

SE: Standard error

SNP: Single nucleotide polymorphism

Sp5: T sphaerococcum var. Sp5

SSD: single seed descent

SSR: Simple sequence repeat (Microsatellite marker)

STS: Sequence-tagged site

Tg: Tenacious glume

μM: Micromoles per litre

USDA: United States Department of Agriculture

v/v: volume/ volume

v/w: weight by volume

CHAPTER ONE

Introduction

Overview

Land races often harbour a significant amount of the available genetic diversity for a crop species. Landraces have been used in the successful breeding of wheat and rice (Swaminathan, 2006; Acquaah, 2007). *Triticum sphaerococcum* (2n = 6x = 42) is a land race characterised by short and strong culm, hemispherical glumes, hemispherical grains with a shallow crease, a compact head and short awns. This land race is early flowering, resistant to yellow rust caused by *Puccinia striformis* and moderately resistant to drought (Percival, 1921). Sphaerococcum wheat also has a higher protein content compared to *T. aestivum*. The hemispherical grains of sphaerococcum with a shallow crease yield more high quality flour (white flour) in comparison to long grains with a deep crease (Singh, 1946).

Bread wheat is the most widely grown cereal crop providing a significant part of the world's dietary protein and starch (Martin et al. 2006). The Food and Agriculture Organisation (FAO) has estimated a record production of 2095 million tonnes of cereals in 2007 /08, an increase of 4.8 percent over the 2006/07 period. The projected harvest included 621.4 million tonnes of wheat (FAOSTAT). Much of the wheat produced in the USA, Canada, Argentina, the European Union and Australia is traded across the world, making it the major food crop controlling global food security (FAOSTAT).

It is evident from the FAOSTAT that climate was the major limiting factor for cereal production in 2006. In the future, a shortage of arable land and a reduction in yield improvements for new varieties may become added constraints to meeting the demand for increased grain production (Rajaram, 2005). Although arable land area is limited, grain yield, yield sustainability, grain quality and resistance to biotic and abiotic stresses can be improved through breeding (Swaminathan, 2006). Breeding for crop improvement started with mass selection. Targeted breeding to produce varieties with superior traits followed the rediscovery of Mendel's principles of genetics in the early 1900s. Despite the doubling of the world's population in the last three decades, food production has coped with population growth (Acquaah, 2007). For example, global wheat production increased at 2% per annum from 1961-1990 (Reynolds and Borlaug, 2006) and a 92% increase in the rice production was achieved

in Asia during this period (Swaminathan, 2006). Norman Borlaug and his research team used the dwarfing genes from the Japanese wheat var. 'Norin 10' to breed high yielding wheat cultivars in the 1960's and 70's. This wheat breeding effort culminated in the "Green Revolution" (Swaminathan, 2006). The *Rht* genes that confer lodging resistance, responsiveness to added nitrogen fertiliser and greater tillering ability were critical for improving the wheat yield/ unit area of land. By 2020 the human population may reach 7.5 billion and the demand for cereal grains may increase by 40-50% (Swaminathan, 2006). Plant breeding efforts have not only improved the yield but also added resistance to biotic and abiotic stresses (Sleper and Poehlman, 2006). Marker assisted selection and genetic engineering have the potential to fast track molecular breeding for crop improvement (Patnaik and Khurana, 2001; Kuchel et al. 2007).

Wheat production was seriously affected by drought in 2005 / 06 (FAOSTAT). Rust, caused by *Puccinia striformis,* is also a significant disease causing considerable loss of wheat production (Suenaga et al. 2003). Consequently, understanding the genetics and physiology of sphaerococcum wheat is important if valuable traits such as resistance to rust and drought, high protein content and hemispherical grains with a shallow crease are to be transferred from this wheat to bread wheat. However, very little information is available on the biology of round grain development, the physiology of its short stature and the inheritance of the complex sphaerococcum trait. The sphaerococcum trait inherits in a non-Mendelian fashion (Sears 1946, 1947) but the mechanism of this inheritance is not clearly understood (Bicknell and Josekutty 2006). This study was carried out at the New Zealand Institute of Crop & Food Research (CFR) at Lincoln, New Zealand to explore the physiology and genetics of the sphaerococcum wheat. This project was funded by the Foundation for Research, Science and Technology (FRST), and many wheat industry partners in New Zealand.

This introductory chapter provides a brief review of the evolution of wheat, its domestication and breeding. Special emphasis is given to the *Rht* genes and their significance in wheat breeding, and the genetics and physiology of sphaerococcum wheat. The chapter concludes with the hypothesis, aims and objectives of this study.

1.1. Origin and evolution of wheat

Wheat (*Triticum* spp.) are members of the family Poaceae. *Triticum monococcum* (2n = 2x = 14, A^mA^m) is the cultivated einkorn wheat. About 4% of the cultivated wheat is the tetraploid, durum wheat (2n = 4x = 28, A^uA^uBB) used for making pasta, macaroni and biscuits. Bread wheat (*T. aestivum,* 2n =6x = 42, A^uA^uBBDD) makes up 96% of the currently cultivated varieties. Being an allopolyploid with A, B and D genomes, the bread wheat genome is large (approximately 16000 Gb) and complex (Gill et al. 2004). *T. timopheevii* (2n = 4x = 28, A^uA^uGG) and *T. zhukovkyi* (2n = 6x =42, $A^uA^uGGA^mA^m$) are cultivated in the Transcaucasia region (Golovina et al. 2007). The phylogeny of wheat and possible origins of the sphaerococcum wheat are presented in Fig. 1.1.

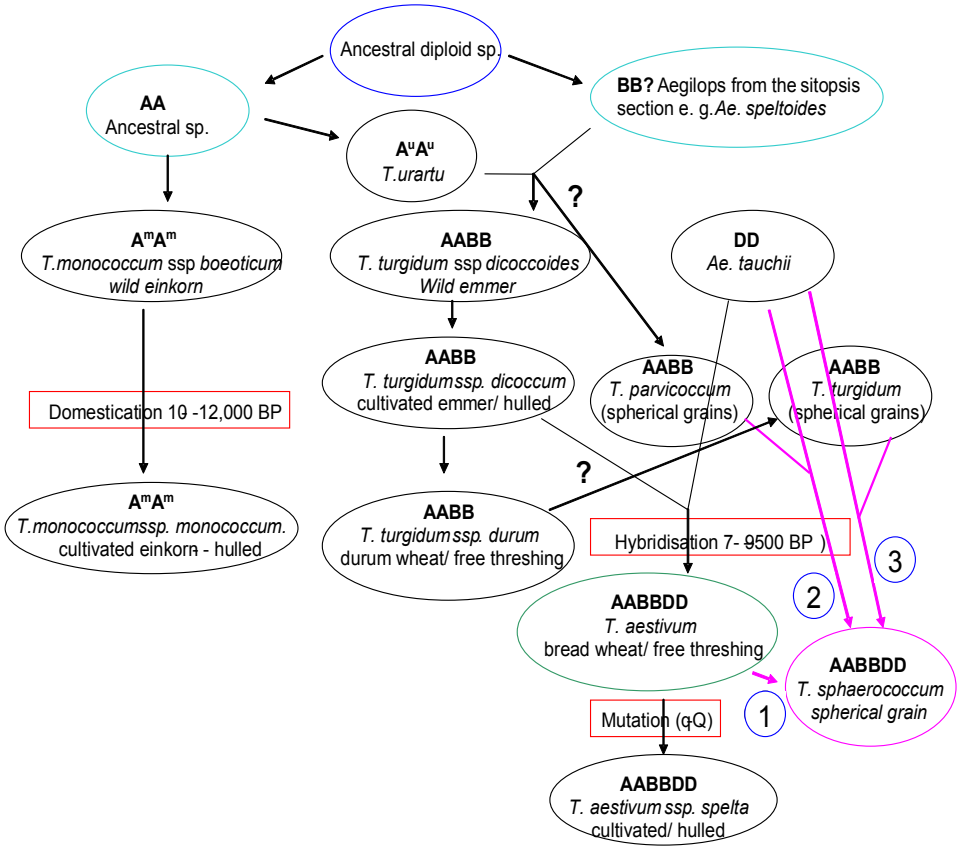


Fig.1.1. Phylogeny of wheat and possible origins of *T. sphaerococcum*.

Possible origins of sphaerococcum wheat (1) through a mutation of the *T. aestivum,* (2) a hybridisation between *Ae. tauchii* and round grained *T. parvicoccum* or (3) a hybridisation between *Ae. tauchii* and round grained *T. turgidum* are shown in the diagram. (Modified from Chantret et al. 2005)

The origin and evolution of wheat has been studied and discussed for nearly a century (Percival, 1921; Kihara, 1944; McFadden and Sears, 1946; Mac Key, 1966; Tsunewaki et al. 1991; Dvorak et al. 1993, 1998; Provan et al. 2004; Feldman and Levy, 2005; Golovina et al. 2007). Wheat originated from a common ancestor that diverged to other cereals such as maize, rice, barley, rye and sorghum about 65 million years ago (Gill et al. 2006). The Pooidae and Panicoideae subfamilies diverged about 60 million years ago. The diploid *Triticum* and *Aegilops* progenitors of A, B, D, G and S genomes all radiated approximately 2.5 and 4.5 million years ago (Huang et al. 2002 a). Diploid wheat (2n = 4x = 28) originated nearly 500,000 years ago and *Triticum aestivum* L. (2n= 6x= 42) is of recent origin, about 8,000 years ago (Gill et al. 2004).

1.1.1. Origin of the A genome

Kihara in 1924 hypothesised that *T. monococcum* is the progenitor of tetraploid wheat and that it contributed the A genome in other polyploid wheat (Mori et al. 1997). More recently, Konarev et al. (1979) argued that the A genome progenitor of *T. timopheevii* may also be *T. monococcum*. However, analysis of wheat chromosomes using Giemsa C-Banding (Gill and Kimber, 1974), analysis of isozymes of α- amylase (Nishikawa et al. 1992), RFLP analysis of repeated DNA (Dvorak et al. 1993), RFLP of the gene coding for the protein disulphide isomerase (Ciaffi et al. 2000), and RFLP of three RbcS subfamilies (Galili at al. 2000) identified *T. urartu* as the source of the A genome in hexaploid wheat. However, Zhang et al. (2002) regarded *T. monococcum* as the source of the A genome from their studies using internal transcribed spacer (ITS) sequences of nuclear ribosomal DNA. After analysing the genome of diploid wheat *T. boeoticum* (2n = 2x= 14, A^bA^b), the progenitor of *T. monococcum* (A^mA^m) and its relative *T. urartu* (A^uA^u) plus tetraploid and hexaploid wheat using AFLP and haplotype analysis, Kilian et al. (2007) concluded that the source of the A genome in durum wheat and aestivum wheat was *T. urartu*. Studies by Dvorak et al. (1988, 1993), Allaby and Brown (2003) and Galili et al. (2000) concluded that *T. urartu* was also the more probable source of the A genome in *T. timopheevii*. Golovina et al. (2007) also supported this view.

1.1.2. Origin of the B and G genomes

There is no general agreement on the source of the B genome in polyploid wheat. Sarkar and Stebbins (1956) and Kerby and Kuspira (1988) considered the *Sitopsis* section of the genus *Aegilops* to be the source of the B genome. Studies on the chloroplast and mitochondrial DNA as well as nuclear DNA of the diploid, tetraploid and hexaploid wheat and *Aegilops* species have concluded that the source of the B genome in tetraploid (wild emmer wheat) and hexaploid (*T. aestivum*) wheat is *Ae. speltoides* (Dvorak et al. 1998; Blake et al. 1999; Ishii et al. 2001; Zhang et al. 2002). Kilian et al. (2007) carried out AFLP analysis using 379 markers on 480 *Aegilops* lines that included all the diploid progenitors of tetraploid and hexaploid wheat. Results of the study were further subjected to haplotype analysis. From these results they concluded that *Ae. speltoides* was the likely source of the B genome found in tetraploid and hexaploid wheat. This study also supported the diphyletic origin of *T. timopheevii* as proposed earlier (Zhang et al. 2002). Haplotype analysis confirmed the AFLP analysis that *Ae. speltoides* is the source of the B genome in the AABB tetraploid wheat and the G genome in the AAGG tetraploid wheat (Golovina et al. 2007).

1.1.3. Origin of the D genome

Kihara (1944) and McFadden and Sears (1944) through independent research artificially synthesised hexaploid wheat by crossing the cultivated Emmer wheat *T. turgidum ssp. dicoccum* (2n = 4x = 28) with *Ae. tauchii* Coss. / (*Ae. squarrosa* 2n = 2x = 14) demonstrating that *Ae. tauchii* is the likely source of the D genome in *T. aestivum* (AABBDD, 2n = 6x = 42). Observations by McFadden and Sears (1946) that hybrids of synthetic and natural hexaploid wheat exhibited complete pairing between homologous chromosomes at meiosis further strengthened their hypothesis. Giemsa C- banding studies (Gill and Kimber, 1974) also supported this hypothesis. From detailed genetic analysis of *Ae. strangulata*, *Ae. tauchii* and *T. aestivum* using restriction fragment length polymorphism (RFLP) analysis, Dvorak et al. (1988, 1993) concluded that *Ae. tauchii* was the likely contributor of the D genome to *T. aestivum*. Based on the restriction site variation of low-copy DNA, Talbert et al. (1998) suggested that the ancestral hexaploid wheat may have formed as a result of multiple hybridisation events involving *Ae. tauchii* and tetraploid wheat. Sequencing

studies with the *Xwye* locus of the D-genome encoding ADP-glucopyrophosphorylase and the *Gss* locus coding for granule-bound starch synthase (Caldwell et al. 2004) in 564 lines of *T. aestivum* supported the hypothesis of Talbert et al. (1998). Recent studies by Kilian et al. (2007), who used 480 wheat lines and AFLP analysis plus haplotype analysis, agreed that the D genome was contributed by *Ae. speltoides* and that there had been more than one hybridisation event in the evolution of polyploid wheat. Golovina et al. (2007), who analysed the *trnT-trnL* intergenic spacer and *trnL* intron regions of the chloroplast in a large number of accessions of different wheat species, goat grass and rye germplasm from a wide geographic area, reached a similar conclusion.

1.1.4. Origin of *Triticum sphaerococcum* Perc.

Percival (1921) awarded species status to *T. sphaerococcum* (AABBDD, 2n = 6x = 42), a land race reported from the Indian subcontinent, based on its distinct morphology (short and strong culm, compact head, hemispherical glumes and hemispherical grains). Being a land race that is not currently under cultivation, it has not been studied in detail. *T. sphaerococcum* readily hybridises with *T. aestivum* producing fertile hybrids, suggesting its close relationship with *T. aestivum* (Ellerton, 1939; Sing 1946; Sears 1947; Schmidt et al.1963). Ellerton (1939) reviewed the origin and distribution of *T. sphaerococcum* to conclude that this wheat originated in the Indian subcontinent. Archaeological investigations found sphaerococcum grains at Mohenja Daro, a centre of the ancient Indus valley civilisation dating back to 9000-10,000 BP strengthening its proposed origin in the subcontinent. Schmidt et al (1963) reported the occurrence of spontaneous mutant hexaploid wheat that resembled *T. sphaerococcum*. Schmidt and Johnson (1963) reported sphaerococcum-like tetraploid wheat among an accession obtained from China. Since there were no previous reports of sphaerococcoid tetraploid wheat, they concluded that it may be a mutant occurring at very low frequencies among the tetraploid wheat. Further studies (Schmidt and Johnson, 1966) revealed that this mutant has an allele that is semi-dominant in nature unlike the sphaerococcum allele *sp*.

Loss of whole chromosomes, as well as chromosomal mutations such as translocations, deletions and duplications, are tolerated by polyploid wheat species. Gene silencing occurs in wheat through non-specific down-regulation of genes and

down-regulation of genes in an orthologue-dependent manner. Activation of specific genes is another method of gene regulation in hexaploid wheat (He et al. 2003). Genome modification may be caused by retrotransposons inserting within or nearby to a gene (Gu et al. 2004). Multiple, independent hybridisation events have lead to the formation of different polyploid wheats (Zhang et al. 2002; Caldwell et al. 2004; Kilian et al. 2007). Bread wheat can be mutated to a sphaerococcum-type (Swaminathan et al. 1963; this thesis). It is also possible to get bread wheat-type mutants from *T. sphaerococcum* through mutation (this thesis). From the foregoing discussion there are three possibilities that may have resulted in the formation of *T. sphaerococcum* as shown in Fig. 1.1. It is possible that a mutation in *T. aestivum* resulted in the formation of *T. sphaerococcum* (1)*; T. sphaerococcum* evolved from a hybridisation between the *sphaerococcum*-type tetraploid wheat *(T. parvicoccum)* and *Aegilops* sp. (2); or a distinct hybridisation event between spherical grained mutant *T. turgidum* and *Ae. tauchii* resulted in the formation of *T. sphaerococcum* (3). Possibility number one, discussed above, appears to be more realistic because we have also independently generated *T. sphaerococcum* by mutating *T. aestivum* as reported by Swaminathan et al. (1963) and generated aestivum-type wheat by mutating *T. sphaerococcum*.

1.2. Domestication and spread of wheat cultivation

The transition of humans from hunter-gatherers to agriculturists started in the Near East about 12,000 BP. Agriculture then spread from the Near East to Europe, Asia, Africa and rest of the world (Salamini et al. 2002). Between 10,000 and 5,000 years ago, quite independently, domestication of different crop plants and animals took place in different parts of the world (Smith, 2001). Possibly the climate change '*the Younger Dryas*' a period of cold and dry climate experienced in the late Pleistocene from 12,200-11,000 BP, might have changed the human life style from a hunter-gatherer to that of agriculturist (Salamini et al.2002; Harris, 2005).

Detailed investigations by botanists, archaeologists and geneticists have identified the 'Fertile Crescent' as the centre of origin and the site for domestication of wheat. The Fertile Crescent lies within the Euphrates and Tigris basin in the Near East, covering parts of Iraq, Iran, Syria, Israel, Jordan and Lebanon (Heun et al. 1997; Martin et al. 2006). From an exhaustive study of cultivated wheat and its wild progenitors from the Fertile Crescent and other parts of the world, Heun et al. (1997)

concluded that *Triticum monococcum ssp. boeoticum* from the Karacadag Mountains in south east Turkey is the progenitor of cultivated einkorn wheat. Combining their genetic evidence with the archaeological evidence from early agricultural settlements in the region they hypothesised that the domestication of wheat began near the Karacadag Mountains in the Fertile Crescent.

Heun et al. (1997) characterised the agronomic and taxonomic traits of 1362 lines of einkorn wheat. They analysed a representative sample of 338 lines from the 1362 characterised lines using 288 amplified fragment length polymorphism (AFLP) markers which are dominant DNA markers. These 338 lines included 68 *T. m. monococcum* lines from different countries, 9 *T. m. aegilopoides* from the Balkans, 194 *T.m. boeoticum* from the Fertile Crescent and 67 *T. m. boeoticum* lines from Turkey, the Caucasus Mountains and Lebanon. Results of the analysis indicated a close relationship among the cultivated einkorn wheat and to *T. m. aegilopoides*. *T. m. monococcum* and *T. m. aegilopoides* showed close phylogenetic attachment to *T. m. boeoticum* lines from the Karacadag region. This study also confirmed the monophyletic origin of the cultivated einkorn wheat. From the genetic relationship of the *T. m. aegilopoides* with the cultivated einkorn wheat and the signs of domestication evident in them, the authors recognised *T. m. aegilopoides* as the intermediate form between the wild einkorn (*T. m. boeoticum*) characterised by small grains and shattering ears and the cultivated einkorn (*T. m. monococcum*) with larger grains and intact ears. The rich diversity of wheat and barley along with pulses, especially the unique diversity of chick pea and several fruits in the region, also strengthened their hypothesis. The diversity of crop plants and small animals used as food in the region may have assisted humans to adapt to agriculture when the 'Younger Dryas' caused food shortages in the region (Harris, 2005). Cultivated wheat seeds dating back to 12600-11200 BP were found at the archaeological sites of Cafer Hoyuk (de Moullins, 1993- cited in Heun et al. 1997) and wild and cultivated seeds from 12,500 to 11,700 BP at Cayonu (van Zeist and de Roller, 1991/92- cited in Heun et al. 1997) near the Karacadag region. Archaeological evidence from Abu Hureyra (Hillman et al. 1989 – cited in Heun et al. 1997) also suggested domestication of wheat in this region took place around 12,800-12,500 BP.

Jones et al. (1998) citing (Smith, 1994) argued that the site of domestication of wheat may be in the Jordan valley because domesticated einkorn, emmer and barley appear between 13,000 and 12,000 BP in the archaeological sites at Jericho, Nativ

Hgdud, and Gilgal in the Jordan basin. They also argued that in the case of maize in Mexico and rice in China the places of domestication are established to be away from their current centres of diversity. Hole (1998) on the other hand suggested that Abu Hureyra on the middle Euphrates of Syria which is also currently a centre of diversity for einkorn wheat may be a more probable region for the domestication of wheat than the Karacadag Mountains. His argument is based on the effect of the 'Younger Dryas' and possible effects of it in the following years. He further suggested that this climate change may have resulted in the development of the Mediterranean climate that favours the growth of annual crop plants including cereals and pulses that are very diverse in the region. However, noted archaeologists, Nesbitt and Samuel (1998) supported the hypothesis of Heun et al (1997) based on archaeological evidence. They cited the shortcomings of the carbon dating technology that was used to determine the age of biological samples from archaeological sites in the region and coupled the botanical evidence and the genetic evidence in supporting the hypothesis of Heun et al. (1997). Discussing the origin of agriculture, Lev-Yadun et al. (2000) agreed with the hypothesis on the domestication of wheat in the Fertile Crescent based on archaeological evidence.

Tanno and Wilcox (2006) studied the time taken to domesticate wheat by analysing the ancient, charred spikelets of wheat collected from four archaeological sites in Syria and south eastern Turkey using carbon dating. The oldest collections dated back to 10,200 BP and the youngest to 6,500 BP. Numbers of domesticated spikelets and terminal spikelets increased as the age of the collection sites decreased. About 20% of the spikelets were of the domesticated type in the collections from 10,200 BC site in Quaramel compared to more than 80% domesticated spikelets observed in the collections from Kosak Shamali dating back to 6,500 BC suggesting that it took over a millennium to effect the domestication of wheat.

The domestication history of the polyploid wheat is complicated by the disputed origin at different sites as evidenced by archaeology and genetics. Wild tetraploid wheat *T. dicoccoides* (AABB) and *T. timopheevii* (AAGG) and *T. araraticum* (AAGG) have a brittle rachis and larger grains (Dvorak et al. 1993; 1998). Domesticated emmer wheat *T. turgidum ssp. dicoccum* and *T. turgidum ssp. durum* with genomes (AABB) are free threshing with non-brittle rachis more suited for harvesting and threshing. Until the early Bronze Age, emmer was the major crop in the Fertile Crescent (Bar-Yosef, 1998) and present in the archaeological sites of Tell

Aswad (10,800 BP). Domesticated emmer remains are found at Abu Hureyra from 10,400 BP (De Moullins, 2000 -cited in Salamani et al. 2002). *T. parvicoccum* grains which are naked and small are present in Neolithic sites (Kislev, 1980 -cited in Salamini et al. 2002). *T. durum* had been the major wheat in Egypt and this variety is still cultivated in Ethiopia. Genetic evidence (AFLP analysis) and archaeological evidence suggest that hulled and free threshing lineages have diphyletic origins and separated very early in their evolutionary history (Salamini et al. 2002).

The progenitors of hexaploid wheat include *T. turgidum* and *Ae. tauchii* (McFadden and Sears, 1946). The geographic distributions of *Ae. tauchii* (DD) and *T. dicoccoides* (AABB) were widely separate about 10,000 BC, thus minimising the possibility of this hybridisation event happening naturally. Therefore, a cross between *T. turgidum* (AABB) with *Ae. tauchii* (DD) which had overlapping geographical distribution may have produced the hexaploid wheat (6x = AABBDD). The hybridisation between the domesticated, free threshing *T. turgidum* with *Ae. tauchii* may have happened at the Caspian basin to which anthropogenic expansion of *T. turgidum* took place (Dvorak et al. 1998).

The *Q* gene, that controls the free threshing character is located on the chromosome 5A, while the tenacious glume (*Tg*) gene, which influences the expression of the *Q* gene is located on the chromosome 2D of *Ae. tauchii*. Using QTL analysis Simonetti et al. (1999) demonstrated that the free threshing character and the brittle rachis are not completely controlled by the genes *Q* and *Tg* alone but by the interaction of these genes with some other genes. Simons et al. (2006) sequenced and studied the expression of the Q gene in detail. They discovered that the *Q* gene has great similarity to the AP2 family of transcription factors in *Arabidopsis*. The *Q* allele has a comparatively stronger expression than the *q* allele. They also concluded that the *q* allele is more primitive than the *Q* allele and the mutation from the *q* allele to the *Q* allele happened only once in the course of the evolution of wheat. This new data raises the question whether the mutation from *q* to *Q* happened in the tetraploid or hexaploid wheat. Archaeological evidence suggests that the free threshing tetraploid and hexaploid wheat appeared about the same time and about 1000 years before the appearance of the spelt wheat (Feldman, 2001). The source of the A genome in the polyploid wheat is *T. urartu* (Dvorak, 1993). It is not clear as to which AB tetraploid (*qq /QQ* genotype) was involved in the hybridisation with *Ae. Tauchii* (source of the D genome). Therefore, Simons et al (2006) suggested that it is not

possible to conclude whether the *Q* allele originated in free threshing tetraploid wheat like *T. turgidum* or *T. parvicoccum* which then hybridised with *Ae. tauchii* to produce the hexaploid *T. aestivum*.

Archaeological evidence suggests that the spread of wheat from the Fertile Crescent to the old world occurred between 8000 to 7000 BP. During the Neolithic period the expansion of agriculture took place first northwards along the Lavantine Corridor and subsequently westward into Anatolia (Bar-Yosef, 1998). Wheat cultivation spread from Anatolia to Greece in Europe around 8,000 BP. Wheat spread from there to Italy, southern France and Spain around 7,000 BP. Around the same time wheat spread northward across the Balkans through the Danube Valley to the Rhine Valley. From there it spread to central, western and northern Europe reaching the Netherlands (6,000 BP), England and Scandinavia by 5,000 BP (Feldman, 2001). Emmer and einkorn wheat spread first to Europe. Free threshing tetraploid and hexaploid wheat were introduced to Europe as a mixture around 6,000 BP (Zeven, 1980 -cited in Feldman, 2001). Another line of spread (mainly bread wheat and club wheat (*T. compactum*) was through Transcaucasia and Caucasus around 7,000 BP reaching southern Russia by 6,000 BP. The spelt wheat, originally a weed along with barley and other grasses among cultivated wheat evolved north of the Alps as a new crop around 4,000 BP (Harlan, 1981).

Wheat spread to Africa through Egypt (6,000 BP), Sudan and Ethiopia or from Greece to Crete and to Libya and through southern Italy through Sicily to Tunis, Algeria and Morocco. Einkorn is reported from Morocco but not from Egypt, Ethiopia and North Africa. Interestingly, naked seeded *T. parvicoccum* reached Africa as a mixture with emmer wheat. Durum wheat was established as the major crop in Egypt by 2,300 BP.

Wheat spread to Asia through Iran. Although wheat was cultivated in Iran by 8,000 BP, it was established in western Pakistan only by 6,500 BP and south western Pakistan by 5,300 BP (Jarrige and Meadow, 1980 -cited in Feldman, 2001). Wheat is reported in India from 5,000 BP and it remained a major crop in India from the third millennium BP (Harlan, 1998 as cited in Feldman, 2001). The spread of white grained wheat to China occurred through the Silk Road that runs from Turkestan through Sinkiang to Northern China. Red grain wheat spread from Pakistan through the Punjab plains, Indian subcontinent to Burma finally spreading through Yunnan to the Yangtse Valley. Wheat reached China around 4,500- 4,000 BP. These routes are

confirmed based on the distribution of the (*Ne*) necrosis gene. Wheat reached Japan only around 2,300 BP (Zeven, 1980). Spaniards introduced wheat to the new world when they introduced it to Mexico in 1529 and the introduction to Australia happened in 1778 (Feldman, 2001).

The origin of *T. sphaerococcum* (6x = 42) is not clear but it possibly originated from *T. aestivum* through a mutation at the *S* locus. Archaeological evidence is available for its domestication only from the Indus Valley. This land race is only found in the Indian subcontinent strengthening the view that it originated and was domesticated in the region (Percival, 1921; Ellerton, 1939; Swaminathan et al. 1963; Feldman, 2001).

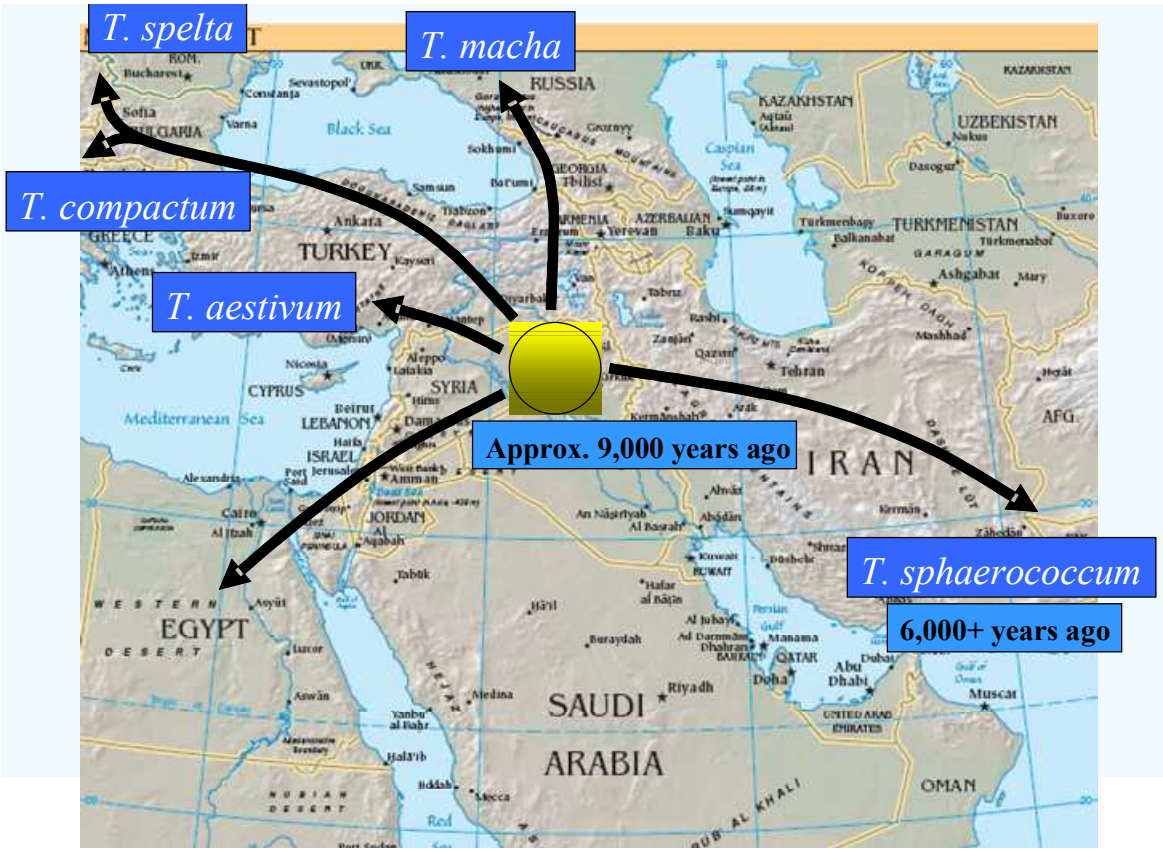


Fig. 1.2 Dispersal and 'speciation' of hexaploid wheat. Hexaploid wheat (*T. aestivum*) originated in the Near East about 9000 years ago and spread to rest of the world. Speciation into *T. compactum*, *T. macha*, *T. compactum* occurred in Turkey and Europe respectively. *T. sphaerococcum* evolved in the Indian subcontinent (Adapted from Bicknell & Josekutty, 2006).

1.3. The significance of the *Rht* genes.

The reduced height genes (*Rht*) control plant form in many wheat varieties. The *Rht* gene confers reduced height and lodging resistance, increased tillering and higher yield to wheat cultivars (Ellis et al. 2004). A highly significant achievement in the recent history of wheat breeding was the introgression of the dwarfing gene (*Rht gene*) from the Norin 10 wheat into American varieties having desirable characters such as disease resistance, adaptability to temperature and photoperiods, which subsequently enhanced the yield and agronomic performance of hybrid wheat (Reynolds and Borlaug, 2006; Swaminathan, 2006). Improved, high yielding semidwarf wheat varieties developed by Borlaug at the *Centro Internationale de Mejoramiento de Maiz Y Trogo* (CYMMIT) or the International Maize and Wheat Improvement Centre, such as Pitic 62, Penjamo 62 and Siete Cerros 66, spread in cultivation across Asia in the 1960s. About the same time the International Rice Research Institute (IRRI) developed lodging resistant, high yielding, semidwarf rice cultivars using dwarfing genes from the Chinese rice 'Dee-gee-woo-gen'. In 1968, Dr. William Gaud, then Director of the United States Department of Agriculture (USDA) termed the revolutionary scale of grain production experienced in Asia from these breeding efforts 'the Green Revolution' (Reynolds and Borlaug 2006; Swaminathan, 2006).

1.3.1. Physiology and genetics of the *Rht* gene.

Reduced height genes (*Rht*) alleles in wheat comprise a homoeoallelic series with pleiotropic effects. *Rht1* and *Rht2* are additive in effect (Allen 1989; Paolillo et al. 1991). *Rht* genes increase wheat yield by reducing plant height and thus favouring a high harvest index. *Rht* genes also increase the number of grains per ear and grain filling because of the increased partitioning of the photosynthetic assimilates into the ear. *Rht3* is known to prevent undesirable pre-harvest sprouting in the field. *Rht* wheat have short and strong culms that are resistant to lodging. *Rht1* and *Rht3* are allelic and they are located on the chromosome 4BS, while *Rht2* and Rht10 located on chromosome 4DS are allelic. These *Rht* genes are associated with an insensitive reaction to applied gibberellic acid (GA_3) (Gale and Youssefian, 1985; Youssefian et al. 1992a and b). The favourable pleiotropic effects of the *Rht* genes have led to their being fixed in 70-80% of modern wheat varieties (Flintham et al. 1997).

Because of the significance of *Rht* genes there has been considerable interest in these genes and their mode of action. Borner et al. (1996) demonstrated that mutant *Rht* alleles are located on chromosome 4B or 4D using nullisomic wheat lines. Peng et al. (1999) isolated and characterised the *Rht* genes from wheat. A comparison of the *Rht* gene with maize *dwarf-8 (d8)* and the *Gibberellin Insensitive (GAI)* genes of *Arabidopsis* revealed that these three genes are orthologues. The functionality of the cloned *Rht* mutant genes was demonstrated in basmati rice transformed using cloned mutant *Rht* genes. The mutant gene product showed modifications at the n-terminus leading to the formation of a dwarf phenotype and insensitivity to applied GA_3 (Peng et al. 1999).

1.3.2. Gibberellin signal transduction

Gibberellins (GAs) form a large group of tetracyclic diterpenoid carboxylic acids, some of which function as regulators of growth and development in plants influencing processes such as germination, leaf expansion, stem elongation, bolting and flowering, seed set and fruit development (Davies, 1995; Fan et al. 2007). About 135 structurally distinct GAs have been characterised from natural sources (http://www.plant-hormones.info/gibberellins.htm). Many of these forms may not be bioactive but are intermediates of the bioactive forms (Yamaguchi and Kamiya, 2000). Because of the significance of this hormone in plant development, its metabolism and the mode of action have been studied at length. The availability of mutants of *Arabidopsis*, maize, rice, barley and wheat having altered responses to GA has helped to understand GA metabolism and signal transduction in higher plants. Perception of the GA signal followed by the signal transduction process leads to modified gene action resulting in a particular phenotype. Recent studies have made excellent progress towards understanding GA signalling mechanisms. These include identification of upstream GA signalling components and *trans*- and *cis*- acting factors regulating GA responsive genes in higher plants (Peng et al.1997; Dill et al. 2001; Gubler et al. 2002; Achard et al. 2003: Fu and Harberd, 2003; Gomi et al. 2004; Swain and Singh, 2005; Nakajima et al. 2006; Shimada et al. 2006; Ueguchi-Tanaka et al. 2007). Ueguchi-Tanaka et al. (2005, 2006) have identified and characterised Gibberellin Insensitive Dwarf1 (GID1) protein as a GA receptor. This GID1 protein isolated from a GA-insensitive dwarf mutant of rice showed specific affinity to bioactive GAs, had a dissociation constant for GA_4 equal to 10^{-7} M and it bound to

SLR1, a rice DELLA protein in a GA dependent manner in yeast cells. Over-expression of GID1 caused a GA-hypersensitive phenotype confirming its identity as a GA receptor.

GA biosynthetic genes are expressed in specific cells and tissue types during development, and their transcript levels are often elevated in rapidly growing regions, such as the rib meristem of shoot apex, elongating internodes, developing anthers and embryo axes (Swain and Singh, 2005). In rice, *GA20ox* and *GA3ox* are expressed in a pattern similar to that of GA signalling genes (Kaneko et al. 2003) further suggesting that bioactive GA may be synthesised at the site of reception. However, recent studies suggest that GA synthesised in the anthers and the developing embryos is probably transported to regulate the growth of other floral organs and fruits. GA synthesised in the embryo during seed germination needs to be transmitted to aleurone cells to induce the expression of hydrolytic enzymes (Kaneko et al. 2003; Ogawa et al. 2003).

Data from studies with *Arabidopsis*, barley, *Brassica*, grape, maize, rice and wheat suggest that the DELLA proteins are the regulators of the GA signalling in higher plants (Thomas and Sun, 2004). The DELLA protein functions as a repressor. Rice and barley have only one DELLA protein each (*SLENDER RICE1* [*SLR1*] and (*SLENDER1* [*SLN1*] respectively. Although, five DELLA protein genes are identified in *Arabidopsis, GA-Insensitive* (*GAI*) and *RGA are* the major GA repressors during vegetative growth, *RGL2* plays a major role during seed germination, while *RGA*, *RGL1* and *RGL2* together modulate flower development (Lee et al. 2002; Tyler et al. 2004; Cheng et al. 2004).

During the upstream regulation of GA, DELLA proteins are degraded by the ubiquitin-proteasome pathway (Sun and Gubler, 2004). In the rice (GA-INSENSITIVE DWARF2 [GID2] and in *Arabidopsis* (SLEEPYF [SLY1) F-box proteins mediate this degradation as part of the E3 ubiquitin ligase SCF [Skp1-Cullin-F-box] complex (McGinnis et al. 2003; Sasaki et al. 2003). In potato PHOTOPERIOD-RESPONSIVE1 (PHOR1), which shows similarity to U-box ubiquitin E3 ligase may be involved in the degradation of the DELLA proteins (Monte et al. 2003). The chromatin –remodelling factor PICLE (PKL) may also function as a positive regulator of GA response (Thomas and Sun, 2004). Negative regulators such as SPINDLY (SPY) of *Arabidopsis,* barley and *Petiunia* are O-linked *N*-acetylglucosamine transferases. These are believed to modify and activate DELLA proteins (Olszewski et al. 2002).

The GA inducible transcription factor GAMYB is a part of the downstream component of the GA response. This transcription factor promotes amylase expression in germinating barley seeds and its homologues have been identified in other species (Lovegrove and Hooley, 2000). Tsuji et al (2006) conducted microarray analysis with Osgamyb mutant rice and concluded that OsGAMYB is involved in the regulation of almost all the GA-regulated genes in addition to the α-amylase genes in the aleurone cells. They further demonstrated that either OsGAMYBL1 or OsGAMYBL2 can compensate for this function in the Osgymb mutant. In anthers, many of the GAMYB-regulated genes contain GARE-like elements and some do not contain pyrimidine boxes and TAT boxes (Tsuji et al. 2006). A study using an electrophoresis mobility shift assay has shown that OsGAMYB interacts with the GARE like sequences in the promoters of GAMYB-regulated genes expressed in the anther in a similar manner to the GARE sequence in the promoter of the aleurone expressed *RAmy1A* gene (Tsuji et al. 2006).

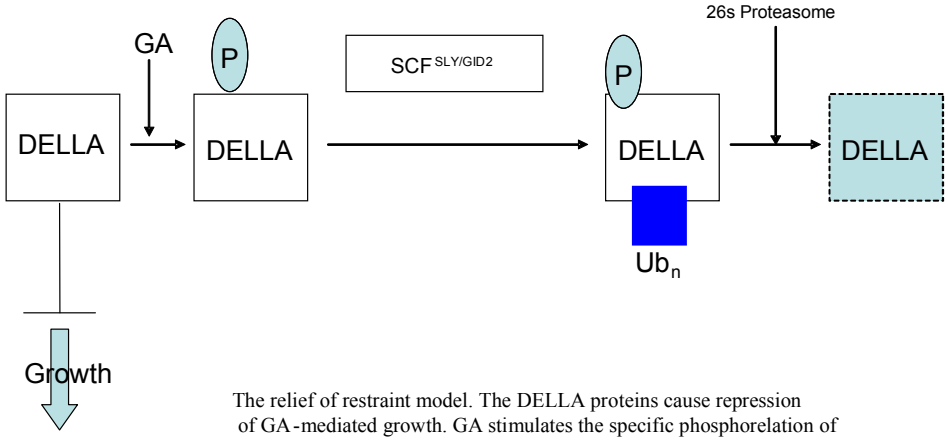
DELLA proteins derive their name from the fact that at the N-terminus of these proteins there are five amino acids whose one letter code is DELLA. At least four of the DELLA proteins identified in *Arabidopsis namely* GAI, RGA, RGL1, RGL2 have GA signalling properties (Peng et al. 1997, Dill and sun, 2001; Tyler et al. 2004). The gai protein is a mutant form of GAI, a DELLA protein. The gai protein is deficient in 17 amino acids found at the N-terminus of the GAI protein. The mutant Rht alleles of semidwarf wheat (*Rht-Bib* and *Rht-D1b*) as well as the *d8* allele of maize code for proteins which are similar to gai (Peng et al. 1999).

DELLA proteins are repressors of growth. The repression of growth caused by DELLA proteins can be overcome by GA. The altered-function mutant gai protein is a repressor of growth that shows lower response to GA. As Rht proteins are similar to gai proteins their mutant forms may be acting in a similar way (Peng et al. 1999; Dill and Sun, 2001; Tyler et al. 2004). Recent studies (Dill et al. 2004; Fu et al.2004) demonstrated that the GAI protein disappears from the nucleus following treatment with GA. Therefore, the DELLA protein bound to the DNA may be destabilised by GA while overcoming the growth repression caused by DELLA proteins. The GAI protein may be easily destabilised by GA, but the mutant gai proteins and proteins coded by mutant *Rht* genes may be more resistant to the GA mediated destabilisation (Alvey and Harberd, 2005).

Selected target proteins are destroyed by the proteasome, which is a highly conserved multi protein complex (Sullivan et al. 2003). Selective targeting of proteins by the proteasome is achieved by poly ubiquitination of the target protein (Vierstra, 2003). The observation that specific inhibitors of proteasome function blocked the GA-induced disappearance of the barley SLN1 protein and also blocked the GA response of barley (Fu et al. 2002) supports the view that the proteasome is involved in this response. Recent studies have identified components of proteasomes that specifically interact with the DELLA proteins of *Arabidopsis* and rice (Mc Ginnis et al. 2003; Sasaki et al. 2003; Dill et al. 2004; Gomi et al. 2004). The interaction of the proteasome complex with its substrate is achieved via phosphorylation (Busino et al. 2003). The rice DELLA protein SLR1 is phosphorylated in response to the GA signal (Sasaki et al. 2003).

Alvey and Harberd (2005) proposed the relief and restraint model model (Fig. 1.3) for DELLA mediated GA signalling.

Fig 1.3 The relief of restraint model for GA3 signal transduction



The relief of restraint model. The DELLA proteins cause repression of GA-mediated growth. GA stimulates the specific phosphorelation of the DELLA proteins that in turn promotes an interaction between the DELLA proteins and the SCF $^{SLY/GID2}$ E3 ubiquitin ligase complex. subsequent polyubiquitination (Ubn) causes the DELLA proteins to be recognised and degraded by the 26s proteasome. Elimination of the DELLA proteins permits growth to proceed.

Adopted from Alvey and Harberd, 2005

DELLA suppresses the transcription required for the GA response by attaching to the DNA. GA causes the destabilisation of the DELLA protein through a proteasome mediated destruction of DELLA proteins. The proteasome mediated DELLA destruction requires phosphorylation and ubiquitination steps. The destruction of

DELLA proteins lead to the derepression of growth. Mutant DELLA proteins such as gai and mutant Rht proteins are less susceptible to the destruction by proteasomes, thus they continue to repress growth even in the presence of GA.

The DELLA protein is an integral component of GA signalling as well as auxin (Fu and Harberd, 2003) and ethylene (Achard et al. 2003; Vriezen et al. 2004) signalling. Fu and Harberd (2003) demonstrated that auxin from the shoot apical meristem controls the growth of roots by influencing the response of root cells to GA. They showed that blocking the polar auxin transport in *Arabidopsis* from the apex to the roots stopped the root growth controlled by GA. This altered response to GA resulted from the increased stability of DELLA proteins in the auxin-deficient plants. Recent evidence suggests that there is interplay of ethylene in the GA signalling. The GA mediated degradation of GFP-RGA complex, a putative transcriptional regulator that repress gibberellin (GA) signaling is delayed by ethylene in *Arabidopsis*. Roots lacking *GAI* and *RGA* are relatively resistant to the growth-inhibitory effects of ethylene. Lack of the ethylene signalling kinase, CTR1, causes a constitutive delay in GA induced GFP-RGA disappearance in *Arabidopsis* (Achard et al. 2003; Vreizen et al. 2004). These lines of evidence demonstrate that there is no GA signalling in isolation but that it is happening as a complex of plant hormone signalling and DELLA proteins may play an integrative role in the plant hormone signal transduction.

1.4. Plant breeding

Acquaah (2007) defined plant breeding as 'the branch of agriculture that focuses on manipulating plant heredity to develop new and improved plant types for use by society'. Breeders have developed plant varieties with higher yield, resistance to diseases and pets, abiotic stress such as cold, heat, drought and salinity tolerance. Molecular breeding assisted by marker assisted selection and genetic engineering has accelerated improving the shelf life (tomato) and nutritional qualities (rice, corn) in food crops. Crop yield depends on the genotype of the plant, the growth environment and the interaction of the genotype with the growing environment. Therefore, improvement in the agronomic practices such as proper irrigation, application of fertiliser and crop protection by the chemical control of pests and diseases also helped to increase crop production in the recent past. Improving yield, nutritional quality,

resistance to biotic and abiotic stress through conventional and molecular breeding will remain objectives of plant breeding in the coming years (Reynolds and Borlaug, 2006; Swaminathan, 2006).

1.4.1. Wheat breeding

Scientific breeding of wheat started in the early 1900s following the rediscovery of Mendelian principles. Prior to the understanding of the genetic principles crop improvement took place through mass selection from the land races grown by farmers (Bell, 1987). Johanssen's discovery of the concept of pure lines coupled with the understanding of the basic principles of genetics paved the way for scientific plant breeding (Lupton, 1987). Wheat is a naturally self pollinating crop bringing about homozygosity unless contamination results from rare (> 1%) cross pollination (Griffin, 1987). Purposeful hybridisation is carried out to bring about desirable variability in a wheat population. Three traditional breeding methods applied to wheat include the bulk method, the pedigree method and the back crossing method. With the advance of technology, tissue culture and doubled haploid production, marker assisted selection; genomics and genetic engineering are now employed to accelerate wheat breeding (Gupta et al. 1999; William et al. 2007).

1.4.2. Methods in wheat breeding

Over the years, several methods such as bulk population breeding, single seed descent, pure line selection, pedigree method, back cross breeding and marker assisted selection (MAS) were developed for wheat breeding. As this thesis is related to wheat genetics and not to wheat breeding as such, only the doubled haploid production and its application to wheat breeding are elaborated while other methodologies are briefly touched upon.

1.4.2.1. Bulk population breeding

In bulk population breeding, desirable parents are hybridised to generate F1 seeds. The F1 seeds are sown as a bulk to raise F2 seeds. From the F2 to the F6 generations the plants are managed as a single large population. Because wheat is a self pollinating crop, 96% purity can be attained by bulking and sowing each successive generation. Selection is then based on progeny performance for the F7 and later generations.

The advantage of bulk population breeding is that a large number of combinations can be managed by a breeder at a reasonable cost with low input into observation and selection. The downside of bulk population breeding is that valuable genotypes may not be noticed from the large population studied by the breeder. In order to address this drawback the bulk population breeding method is modified to include negative or positive selection in the segregating generations.

1.4.2.2. Single seed descent

The single seed descent (SSD) method is a modification of the bulk population method. This method can be used to accelerate the selection and production of new lines (Borojevic, 1990; Sleper and Poehlman**,** 2006). In this method, following the original cross, single seeds from each of the plants are randomly sampled in subsequent generations. Because only single seed is used from each plant, segregating generations can be grown in a small area such as a glass house allowing the study of 2-3 generations per year. According to Cross and Wallace (1994), SSD relies on the assumption that every individual genotype has an equal opportunity of being selected for subsequent plantings and is of equal productive fitness when compared with other genotypes. Seeds may not have equal productive rates and different environments may favour some individuals over others. Therefore, during SSD, certain genotypes may be favoured whilst others are lost due to selection pressures. Study of several cereal lines produced through SSD have shown that a portion of the genotypes are lost when only one seed per plant is sampled and this may result in skewed segregation ratios (Borojevic, 1990; Snape et al. 1992).

1.4.2.3. Pedigree method

In this method, superior types are selected in segregating generations and a record of all parent-progeny relationships is documented. (Jensen, 1988; Slepper and Poehlman, 2006). Selection is started at the F2 in this method. Since the plants at the F2 are highly heterozygous and segregating, a large number of the progeny are screened on an individual plant basis. As wheat is mostly self-pollinated, homozygosity can be achieved in many loci by F3-F4 generations and some family characteristics can be identified by the F4 generation. Therefore, selection of the best plants from these families will be of advantage at this stage. By the F5-F6 generations, most families may achieve homozygosity for many loci and, therefore, selection within families may not be effective any longer. Pedigree records are used at this stage to eliminate all but one member of such closely related families (Jensen, 1988; Slepper and Poehlman, 2006).

In the pedigree method, the selection is based on genotypes as well as phenotypes. Hence, the chance of obtaining superior genotypes is higher in this method than the bulk selection method (Borojevic, 1990). The down side of the pedigree method is that it is time consuming and laborious. It is possible that the effects of heterosis and dominance can mask the genotypic value of a plant over many generations. The pedigree method is used more frequently in self-pollinated crops than bulk methods because of the greater chances for selecting superior genotypes (Jensen, 1998; Borojevic, 1990). Singh et al. (1998) proposed that any selection scheme that shifts population frequencies towards desirable phenotypes should result in higher frequencies of superior genotypes. Therefore, the selection of parents is more critical than the type of selection scheme used to make selection in a segregating population.

1.4.2.4. Back cross breeding

The back cross method is applied to add a specific trait or a group of related traits to a particular variety of interest (Jensen, 1998). For example, if a superior parent plant (A) with favourable characters such as high yield and wide geo-climatic adaptability but deficient in resistance to a certain disease is identified, that parent (A) can be used as a recurrent parent in crosses with variety (B) that has resistance to the particular disease. If the disease resistance character is dominant (*RR*) then all the F1 plants will be heterozygous (*Rr*) and resistant to the disease. The F1 will be crossed

with the recurrent parent (A) in the next generation. The progenies of the back cross will segregate at 1:1 ratio for *Rr: rr*. The back crossing will be repeated typically six times. Following this the selected line will be field tested for its superior traits plus the resistance factor following the pedigree method. The back cross method is not normally used alone, rather it is used in combination with the pedigree method. The down side is that this method works well for a monogenic trait but the efficiency decreases with the involvement of more genes controlling the same trait and it is more difficult with recessive traits unless a marker is available.

1.4.2.5. Marker assisted selection (MAS)

Marker assisted selection (MAS) offers the opportunity to use genotype based breeding rather than phenotype based breeding which is more time consuming (Gupta et al. 1999). MAS is increasingly used in wheat breeding because it is more efficient compared to the conventional breeding practices and can be applied to improve a wide range of complex traits. Efficiency and ease of applying MAS is enhanced by the availability of molecular markers and the development of user friendly, high throughput technologies (Gupta et al. 1999). MAS can improve breeding efficiency by carrying out selection not directly on the trait of interest but on molecular markers linked to that trait. Molecular markers are not environmentally regulated. Thus, they are not affected by the growing environment of the plants unlike phenotypic markers. Furthermore, molecular markers can be assessed at any stage of plant growth unlike many phenotypic markers that can be assessed only at certain stages of plant growth (Mohan et al. 1997; Gupta et al. 1999; William et al. 2007; Kuchel et al. 2007c).

Podlich et al. (2004) identified the basic steps for successful application of MAS for crop breeding and improvement as: (i) the creation of a dense genetic map of molecular markers, (ii) the detection of quantitative trait loci (QTL) based on statistical associations between marker and phenotypic variability, (iii) the identification of a set of desirable marker alleles based on the results of the QTL analysis and, (iv) the application and /or exploration of this information to the current set of breeding germplasm to enable marker based selection decisions to be made. William et al. (2007) identified more applications for MAS. Gene pyramiding, which is used to develop crops with several gene combinations to enhance the durability if traits like disease resistance, can be achieved through MAS. In the case of introgressing recessive genes, extra steps of selfing can be avoided and breeding time

can be saved using MAS. MAS also can reduce the cost of screening in cases where conventional means are expensive. In the case of assays for quality traits in seeds the number of seeds available in the early stages of a breeding programme can be a limiting factor. In such cases MAS based on DNA markers can be used to reduce the number of seeds required for the assay.

A number of the molecular markers available for wheat, such as simple sequence repeats/ microsatellites (SSR), sequence tagged sites (STS), restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP) or random amplified polymorphic DNAs (RAPDs), are amenable to large scale applications in molecular breeding. Recently developed expressed sequence tags (ESTs) as well as single nucleotide polymorphisms (SNPs) will be of great significance to MAS in wheat in near future (Somers et al. 2003b; Qi et al. 2004; Raman et al. 2007). The co-dominant SSR markers that have high rates of reproducibility are of great value for MAS in wheat compared to RAPD and RFLP markers tagged to some genes (William et al. 2007).

Recent studies have shown that early generation selection with markers is the most efficient way of enriching the frequency of favourable alleles in segregating progenies (Bonnett et al. 2005; Kuchel et al. 2005). The number of genes tagged using molecular markers and the use of MAS in wheat breeding is steadily increasing. At CYMMIT alone twenty six different markers are used in the wheat programme that utilises MAS (William et al. 2007). Other recent examples of the successful use of MAS in wheat include, but are not limited to, the development of *Bo-1,* a marker for boron tolerance (Jefferies et al. 2000), perfect markers (*Cre1* and *Cre3*) for the cereal cyst nematode resistance (Ogbonnaya et al. 2001), QTL for crown rot resistance (Collard et al. 2005), and markers (*Lr34 and Yr18*) for resistance to leaf and stripe rusts (Lagudah et al. 2006). A SNP marker (*XTc1*) and a **c**leaved **a**mplified **p**olymorphic **s**equences (CAPS) based maker (*XPPO*$_{_L}$DOPA) were identified and characterised as diagnostic markers for selecting low polyphenol oxidase wheat (Raman et al. 2007).

The projected down side of applying MAS for crop improvement is the cost factor (Young, 1999; Koebner and Summers, 2007). MAS may be a more expensive process compared to conventional breeding under most circumstances. The cost of a MAS assay, including the cost of DNA extraction, PCR amplification and gel based separation, was computed to be A$1.09 per assay. This could be reduced to A$0.60

provided multiplexing using the capillary based detection system was used (Fox et al. 2005; Hayden et al. 2005). Globally, most of the wheat breeding is carried out by publicly funded programmes. The self pollinating nature of wheat leads to the stabilisation of open pollinated lines. Consequently, farmers often use a part of their harvest as the source of seed for the next generation of the crop. This, together with the low value situation of a wheat crop makes it unattractive for private breeding companies to deal with wheat breeding. Increased availability of desirable markers and high throughput technologies are leading to increased application of MAS to this crop (Jenkins and Gibson, 2002; Roses, 2002; Somers et al. 2003; Koebner and Summers, 2007; William et al. 2007).

1.4.2.6. Haploids and doubled haploids in wheat breeding and genetics

Doubled haploid production is of great significance to cereal breeding because it can produce fertile homozygous lines in a single generation. This is particularly valuable with recessive traits as they are expressed in a homozygous condition only (Campbell et al. 2000; Pratap et al. 2006; Ushiyama et al. 2007). Anther culture is often more cost effective than using wide hybridization followed by embryo rescue to produce doubled haploids (Snape et al. 1986; Snape and Parker, 1987). However, the recalcitrance of microspores in many cereal genotypes makes it difficult to regenerate whole plants from microspores. Several studies have shown that the recalcitrance can be overcome completely or in part by modifying the culture conditions. Changing the physical state of the medium (liquid/ gel), light conditions (incubation in the dark/ under light), changing the ambient temperature during incubation and irradiating the anthers have modulated the development of microspores into plantlets *in vitro* (Dunwell, 1985; Kasha et al. 1990, Ling et al. 1991). Treatment of microspores with 2-hydroxynicotinic acid, minerals and maltose in the dark improved the yield of wheat plants from cultured microspores (Zheng et al. 2001). Microspore culture of wheat and other cereals may produce a large proportion of albino plants (Marsolaris, 1989). *In vitro* genotypic selection leading to skewed segregation ratios is also possible in the wheat anther cultures (Snape et al. 1992; Bjornstad et al. 1993).

Haploids can be produced from cultured microspores because a certain proportion of the microspores are embryogenic under specific *in vitro* culture conditions (Gilpin, 1996). Pollen grains of cereals that are in the mid-late uninucleate stage of development are best suited for embryogenesis in tissue culture (Dunwell,

1985; Mitchell et al. 1992; Kisana et al. 1993; Pratap et al. 2006). Deviation from this critical development of the pollen significantly reduces the efficiency of embryo development (Dunwell, 1985).Culture of haploid maternal tissue is another possible means for producing haploid plants. Culturing cereal ovaries containing an embryo sac at the appropriate stage of development can yield haploid plants (Dunwell, 1985). The appropriate developmental stage of the ovary is judged by assessing the developmental stage of the microspores to the binucleate stage (Pickering and Devaux, 1992; Sibi et al. 2001). Ovary culture requires vernalisation and the use of complex media and incubation procedures (Mejza et al. 1993; Sibi et al. 2001). Ovary culture of wheat produces fewer plants compared to anther / microspore culture but the proportion of albino plants formed with ovary culture is less than that arising in anther culture (Sibi et al. 2001).

Wide hybridisation for haploid production in wheat

Haploid wheat plants can be obtained by rescuing the hybrid embryo of crosses between wheat and some members of the graminae, because of the selective elimination of non-wheat chromosomes during the development of the hybrid embryo (Knox et al. 2000; Brazauskas et al. 2004; Verma et al. 2005; Chaudhary et al. 2005; Guzy-Wrobelska et al. 2007). Barclay (1975) observed that the hexaploid wheat var. Chinese Spring can be crossed with *Hordeum bulbosum* to produce haploid wheat. However, the sensitivity of the *H. bulbosum* to the dominant, crossability inhibitor genes *Kr1* and *Kr2* restricts the ability of *H. bulbosum* to fertilise ovules of most of the wheat genotypes. Laurie and Bennett (1987) developed the wheat X maize system for haploid production which is not affected by the *Kr1* and *Kr2* genes because maize pollen is insensitive to these genes. A few other members of the graminae such as teosinte (Suenaga et al. 1998), *Tripsacum dactyloides* (Mujeeb-Kazi, 1993), *Sorghum* and pearl millet (Inagaki and Mujeeb-Kazi, 1995) and *Imperata cylindrica* (Chaudhary et al. 2005) can be used to produce haploid wheat through interspecific hybridisation.

Various possible crosses leading to haploid production may pose different challenges. The cross between wheat and *H. bulbosum* is limited by the presence of *Kr1* and *Kr2* genes in wheat. Maize is a better source of pollen because of its ability to cross and produce embryos with different genotypes of wheat. However, the low percentage of embryo formation in the wheat X maize cross is a limiting factor

(Laurie and Bennett, 1989, Matzk and Mahn, 1994; Chaudhary et al. 2005). Several factors can affect the success rate of doubled haploid production through the wide hybridisation method. These include growth conditions of the plants (Campbell et al. 1998, Zheng et al. 2001), quantity, type and method of application of hormones (Matzk and Mahn, 1994; Almouslem et al. 1998; Campbell et al. 1998), method of inoculation (Suenaga et al. 1997), wheat genotype, media and incubation conditions for the rescued embryos (Sarrafi et al. 1994; Almouslem et al. 1998; David et al. 1999; Knox et al. 2000, Chaudhary et al. 2005), pollen source of maize (Zhang et al. 1996; Campbell et al. 1998) and the effect of the pollinator (Garcia-Llamas et al. 2004).

Doubled haploids in genetic studies

The genotypes and genetic ratios of doubled haploid (DH) populations are similar to the ratios found in gametes. Therefore, there are several advantages in using the DH populations over the conventionally used F2 populations for genetic studies (Pauls, 1996).

i) Being homozygous, the traits that are conveyed by recessive alleles will expressed in the DH populations.
ii) DH populations have fewer genotypic classes and larger differences between the classes than F2 populations. Therefore, much smaller DH populations are sufficient to observe all possible phenotypes from a cross. This feature is very useful for mapping studies.
iii) Because the DHs are fixed, they can be used for analysis in following generations without losing gene combinations of interest (Hu, 1996; Pauls, 1996).

Slepper and Poehlman (2006) pointed out the inefficient production of haploids in many plant species and the genetic variability arising during anther culture, leading to genetic instability in DH lines developed from such haploids, as downsides of using DHs in breeding programmes. Guzy-Wrobelska et al (2007) have demonstrated that genetic instability in wheat DH lines was not significantly different from recombinant inbred lines. Recent reports suggest that DH lines are widely used in genetic studies [molecular mapping and QTL analysis] in wheat mainly because of the ease of

producing DH lines in comparison to the production of recombinant inbred lines. Genetic analysis of adult-plant resistance to leaf rust in wheat was recently evaluated using DH lines of wheat (Brammer et al. 2004). Kuchel et al. (2005) hypothesised based on simulation studies that the use of MAS with DHs will be of great advantage to wheat breeding. Verma et al (2005) detected QTLs related to lodging resistance in wheat using a DH population. Wheat DH lines were used to screen for the quality of bread wheat (Kozub et al. 2006). Huang et al. (2006) successfully used wheat DH lines to detect QTLs for agronomic traits like grain yield, plant height, maturity, lodging, test weight and thousand-grain weight as well as the quality traits such as grain and flour protein contents and gluten strength.

1.5. Sphaerococcum wheat (*T. sphaerococcum* Perc.)

Triticum sphaerococcum is hexaploid wheat. It is a land race whose natural populations are found only in the hot and dry climatic regions of the Indian subcontinent. It is semidwarf wheat with strong culms and straight leaves. It has high tillering ability, short ears, short awns, hemispherical glumes and grains. It is reported to be drought resistant and has resistance to leaf rust (Percival, 1921). Percival (1921) raised it to species status and described six varieties of this species namely var. *echinatum*, mihi; var. *spicatum*, mihi; var. *rubiginosum*, mihi; var. *tumidum*, mihi; var. *rotundatum*, mihi and var. *globosum*, mihi. Sphaerococcum grains are hemispherical with a shallow crease and thus yield more white flour than grains with a deep crease. The grain has greater protein content compared to bread wheat (Singh, 1946).

1.5.1. Physiology of sphaerococcum wheat

Interesting features of sphaerococcum wheat include the semidwarf stature, high tillering ability, early maturing habit, hemispherical grains with a shallow crease, resistance to leaf rust and drought. In bread wheat, the semidwarf character is contributed by the mutant *Rht* alleles that make the plants less responsive to GAs (Peng et al. 1999). However, the genetic and physiological basis of the semidwarf phenotype in the sphaerococcum wheat is not known. Percival (1921) reported that the sphaerococcum land races he studied in the UK were moderately resistant to

drought and matured early. Some land races of sphaerococcum wheat studied in India were not resistant to drought (Singh, 1946). Swaminathan et al (1963) hypothesised that the early maturing and drought resistant characters of sphaerococcum wheat as reported by Percival (1921) may have helped the species to establish and thrive in hot and dry regions of the Indian subcontinent that experience a short monsoon season. Because the only two studies on the drought resistance of sphaerococcum wheat are conflicting, a conclusion can't be reached on this character. There are no conclusive investigations made on the physiological basis of the sphaerococcum grain characters, tillering ability or early maturity.

1.5. 2. Genetics of the sphaerococcum wheat

The genetics of sphaerococcum wheat is not clearly understood. Miczynski (1930 - cited in Ellerton, 1939) studied the inheritance of sphaerococcum wheat by crossing it with *vulgare* (synonym – aestivum) wheat. He followed the segregation of the sphaerococcum trait through to the F2 generation. From the observed results he concluded that the complex sphaerococcum characters were inherited as one block, as if it was controlled by one gene. Miczynski denoted *vulgare* wheat as '**SS'** and sphaerococcum as a recessive for the trait with an '**ss'** genotype.

Ellerton (1939) studied the cross between *T. vulgare var. Chinese White* with *T. sphaerococcum var. rubiginosum.* All the F1 progeny in this cross resembled the *vulgare* wheat, although, the dominance of the *vulgare* trait was not complete. In the F2, he observed the segregation of *vulgare* and sphaerococcum phenotypes at an approximately (**1:2:1- SS: Ss: ss)** ratio**.** In the F3, he observed a **3:1** ratio of segregation for **S** and **s** alleles. He noted that most of the **Ss** plants in the F2 could be classified as typically *T. vulgare.* Some heterozygotes were intermediate in character as if they were cross-overs because they had the hemispherical glumes but with long, *vulgare* type grains; with *vulgare* like ears and sphaerococcum like grains and so on. In the F3, however, all the cross-over types proved to be "fluctuants". He then followed up the height factor alone for a cross-over type plant found in the F2 through to the F4 generation. The starting material selected from the F2 was 93.9 cm tall but the average height of the 493 plants derived from this plant in the F4 was only 53.8 cm resembling the sphaerococcum phenotype. Analysing the awn length alone, he noticed that in the F2, there were a lot of intermediate types for the awn length (fully

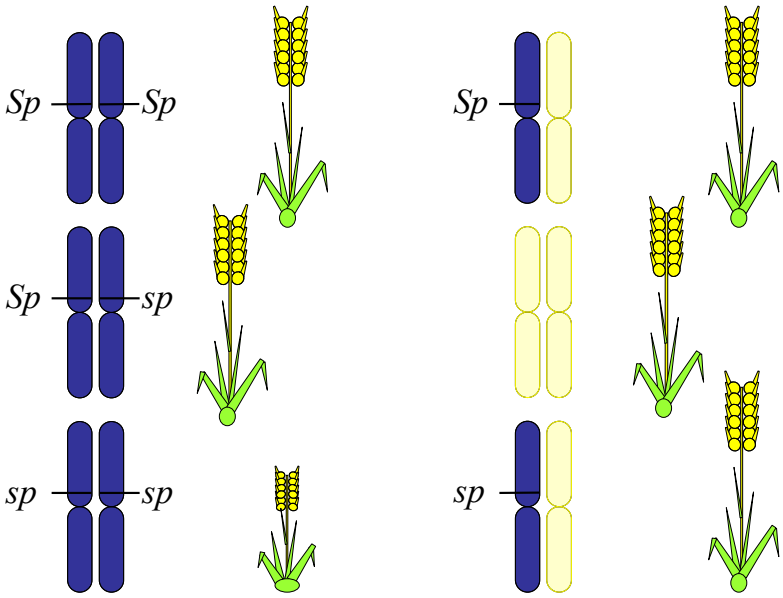
bearded to awn less) among the *vulgare* types and sphaerococcum types. However, in the F3 all the vulgare types had long awns (~ 5cm) while the sphaerococcum types had short awns (~1.5 – 2.0 cm).

Based on these studies Ellerton (1939) hypothesised that "*The two species differ by a single deletion covering several loci, the general characteristics of the mutation suggesting that it is the effect of gene deficiency. Such a deletion would, of course, be preserved intact and would be inherited as a single Mendelian factor*". He also considered explaining the observed genetic behaviour as a result of an inversion too small to permit the frequent formation of chromatic bridges at meiosis, making possible the isolation of a series of point mutations in a short section of the chromosome. Singh (1946) following crosses between sphaerococcum and *vulgare* wheat concurred with the results of Ellerton (1939). He studied the cytological behaviour of sphaerococcum and its hybrid with *vulgare* wheat. Comparing his results with that of others (Percival, 1939; Vakar, 1932; Kihara, 1934; Kihara, 1937 and Lilienfeld and Kihara, 1937 as cited in Ellerton, 1939) he concluded that *T. sphaerococcum* is closely related to the *T. turgidum*, *T. vulgare*, *T. spelta* and *Ae. ovata.*

Sears (1947) studied the inheritance of the sphaerococcum trait by crossing it with 17 different nullisomics of *T. vulgare* var. Chinese Spring. He noticed complete dominance of the *vulgare* trait over the sphaerococcum trait in the F1. He noticed that in one F2, involving chromosome XVI of the D genome, the segregation for sphaerococcum type was not random, but depended upon chromosome number. In this case the disomic plants were sphaerococcum, while the nullisomics resembled *vulgare*. From this observation he concluded that the sphaerococcum gene is located on chromosome XVI (3D). He also concluded that the sphaerococcum gene in double dose produced sphaerococcum effect but in single dose it was relatively ineffective (hemizygous ineffective) as illustrated in Fig. 1.4 This study invalidated the deletion hypothesis of Ellerton (1939).

Schmidt et al. (1963) studied a cross between sphaerococcum-like spontaneous mutants of *T. aestivum* (hard wheat) with *T. sphaerococcum var. globosum.* Although, the sphaerococcoid mutant and the *T. sphaerococcum* variety had similar characteristics, the two genes were not allelic.

Fig 1.4 Summary of the conclusions from the nullisomic analysis by Sears (1947)



The *sp* gene in single dose is ineffective "*hemizygous ineffective*" but in double doses this gene produced the sphaerococcum phenotype.

They suggested a change to the gene symbol for sphaerococcum from sp to ***sp*$_1$** and sphaerococcoid mutant as gene **S*p*$_2$**. In this thesis the gene symbol ***sp*** is used to denote the natural sphaerococcum allele. Schmidt and Johnson (1963) reported the occurrence of a sphaerococcoid mutant among a durum wheat (*T. turgidum ssp. durum*, 2n = 28) accession obtained from China (C.I. 8594) and speculated that this tetraploid wheat may have the mutated ***sp*** gene on the A or B genome. In the same communication they cited a personal communication from B. C. Jenkins, University of Manitoba, Canada, that R. A. Sangeve, from India, who was a graduate student at the University of Manitoba had a similar sphaerococcoid mutant durum wheat that resulted from the cross *T. turgidum ssp. durum var. Gazza* X *T. turgidum ssp. durum var. Baxi 23.* Schmidt and Johnson (1966) hybridised the spontaneous mutant sphaerococcum type durum wheat with normal durum wheat. F1 progeny resembled the normal durum wheat, although, there was partial expression of the sphaerococcum characters. Of the 377 F2 progeny studied, 285 were normal-type and 92 were sphaerococcum- type producing a 3:1 F2 monohybrid ratio. From this result and their previous results (Schmidt et al. 1963; Schmidt and Johnson, 1963) they concluded that the sphaerococcum trait was not restricted to the D genome alone but may be present on the A and B genomes as well.

There are reports of generating sphaerococcoid mutants using chemical mutagens and ionising radiations (Swaminathan et al.1963; Bozzini, 1965; Chopra and Swaminathan, 1966; Gupta and Swaminathan, 1967). The sphaerococcoid mutant derived from the exposure to UV rays or β rays from ^{35}S were allelic to the natural *sp* allele, whereas the mutant produced using the chemical agent ethyl methane sulphonate (EMS) was non allelic to *sp* but similar to the mutant (*Sp*) described by Schmidt et al. (1963). Chopra and Swaminathan (1966) isolated a sphaerococcoid mutant from durum wheat (*T. turgidum ssp. durum,* 2n = 28) using chemical mutagens EMS and hydroxylamine. Gupta and Swaminathan (1967) reported that the mutant sphaerococcoid durum wheat is a dominant mutation (*Sp*) and it is non allelic to the *sp* allele.

Rao (1977) reported that the *s* gene is located close to the centromere (5.7%) on 3Dβ (short arm) of chromosome 3D. Koba and Tsunewaki (1978) mapped the sphaerococcum gene (*s*) in hexaploid wheat using *T. aestivum* cv. Chinese Spring with genotypes (SS or --) and an isogenic marker line with genotype (ss). They mapped the s gene on the long arm of chromosome 3D with a genetic distance of 5.0 ± 0.2% to the centromere. Singh (1987) mapped the sphaerococcum gene (*s*) using var. Pb. C 591 which is monosomic for 3D, *T. aestivum* var. Chinese Spring (red seeded), ditelocentric for 3D long arm and a red seeded variety of *T. sphaerococcum*. He mapped the ***s*** gene to the 3DL at 5.4 cM from the centromere.

Recently, Salina et al. (2000) mapped the induced sphaerococcoid genes *S1*, *S2* and *S3* on chromosomes 3D, 3B and 3A respectively using microsatellite markers. The *S1* locus was mapped close to the centromeric marker *Xgwm456* (2.9 cM) on the long arm and 8.0 cM from the marker *Xgdm72* on the short arm of chromosome 3D. The *S2* gene located on chromosome 3B is tightly linked to two centromeric markers (*Xgwm566* and *Xgwm845*). The *S3* gene is located between *Xgwm2* (5.1 cM) on the short arm and *Xgwm720* (6.6 cM) on the long arm of the chromosome 3A. From the map locations of *S1*, *S2* and *S3* identified close to the centromere on chromosome 3A, 3B, 3D and considering the physical mapping of *s* gene close to the centromere of chromosome 3D (Rao, 1977; Koba and Tsunewaki, 1978). Salina et al. (2000) concluded that sphaerococcum phenotype may be resulting from gene duplication arising out of DNA recombination in the centromeric region. The natural allele of sphaerococcum gene *s* has not been investigated and mapped using molecular markers.

1.6. Hypothesis and objectives

1.6.1. Hypothesis

The genetics and physiology of the complex sphaerococcum trait is poorly understood. If the low yield trait in *T. sphaerococcum* could be separated from the favourable traits, it may be possible to develop superior bread wheat varieties through breeding. Therefore, the following hypotheses were tested in this study.

1. The physiological basis of *T. sphaerococcum* is similar to that of the semidwarf varieties of *T. aestivum*.
2. The unfavourable character (low yield) in *T. sphaerococcum* could be separated from favourable traits (short and strong culm, hemispherical grains with shallow crease) through mutation.
3. *T. sphaerococcum* originated through a mutation in *T. aestivum*.

1.6.2. Aims and Objectives

The first objective was to determine the physiological basis of the lodging resistant, short and strong culms of *T. sphaerococcum.* This was addressed by comparing the growth and development of *T. sphaerococcum* with *T. aestivum* varieties that have known *Rht* genes.

The second objective was to investigate the genetic basis of the complex sphaerococcum trait and to determine whether the trait is controlled by a single gene or more than one gene. A mapping population of doubled haploids developed through wide hybridisation was analysed using molecular markers (SSR and DArT markers) to understand the genetic basis of the complex sphaerococcum trait. GA_3 was also employed as a probe to address this question.

The third objective was to determine the origin of *T. sphaerococcum* and to evaluate the possibility of breaking the sphaerococcum complex into its component traits through mutation. This was addressed by mutating *T. sphaerococcum* and T. aestivum with gamma radiation followed by phenotyping the mutants.

CHAPTER TWO

MATERIALS AND METHODS

2.1. Plant material

Triticum sphaerococcum var. Sp5 (USDA accession # PI352 498) and Otane, a modern bread wheat (*T. aestivum*) cultivar, were the principal plant materials used in this study. Other wheat (*T. aestivum*) cultivars used for the physiological studies included CS971 (*Rht2*), Chuan-Mai 18 (*Rht8*), V MeS3 (*Rht12*), Rasp (*Rht13*), and Icaro (*Rht18*). Sweet corn (*Zea mays*) var. NZ71was used for wide hybridisation with F_1 hybrid wheat (Otane x Sp5) to generate the doubled haploid wheat used for gene mapping.

2.2. Chemicals

All the chemicals used in this study were of analytical grade, purchased from Sigma Chemical Co., USA, Invitrogen, Australia Pty Ltd., Quiagen or other local suppliers. Nursery supplies such as pots, slow release fertilisers and potting mix were procured locally. Grow-lux lights were used in the glasshouse to provide suitable growing conditions (light intensity, temperature and photoperiod) in the environment controlled glasshouses at the University of Canterbury, Christchurch and Crop & Food Research Ltd., Lincoln.

2.3. Seed germination studies

Seeds of Sp5 and Otane were germinated on Whatman # 1 filter paper discs (7 cm) soaked in 10 ml purified water (RO pure) in deep Petri-dishes (10 cm x 2 cm). Seeds were incubated at 22 ± 3˚C in darkness and assessed for germination. Each Petri-dish contained 20 seeds and three replicates were used for every repetition of the experiment. These seeds were visually evaluated after 72 h of incubation.

2.4. Effect of plant hormones on early seedling growth

Seeds of Otane and Sp5 were grown in polycarbonate vessels (12 cm x 5 cm) to compare their growth over a 144 h period. Seeds were grown on filter paper discs soaked in pure water (control) or with aqueous solutions of indole-3-butyric acid (IBA), gibberellic acid ($GA_{3)}$ and 6-benzylaminopurine (BA). The seeds were then incubated at 22-25˚C in a growth room under 96- 100 μmol m^{-2} s^{-1} photon flux density

(PFD) provided by cool, white, fluorescent tubes. IBA and BA were dissolved in 0.1 M KOH solution while GA_3 was dissolved in minimal amount of ethyl alcohol (EtOH) before diluting with purified water (RO pure). These hormones were applied at concentrations of 0, 1.0, 3.3, 10, 33 and 100 μM. The pH was adjusted to 5.7 using 0.1 M HCl or KOH as required before incubation the seeds. Elongation of the shoots (base to the tip of the leaf) was measured at regular intervals from 48 h to 144 h after imbibition.

2.5. Determination of rate of cell division

The rates of cell division in the root tip of Otane and Sp5 were compared using squash preparations according to Berlyn and Miksche (1976). The procedure was carried out in five steps as explained below. (i) The roots of three day old seedlings were fixed in Farmers' fluid (acetic acid: anhydrous ethyl alcohol 3:1(v/v) at 60˚C for 10 minutes; (ii) fixed root tips were heated (without boiling) in 1 N HCl for 5-10 minutes by passing over a spirit lamp flame; (iii) three root tips were transferred to a drop of 45% (w/v) aceto-orcein stain and a drop of 45% (v/v) acetic acid on a clean microscope slide. The slide was warmed over the flame and excess fluid on the slide was removed using a blotting paper. Step iii was repeated twice; (iv) cover slip was placed over the stained root tips and the slide was placed on a blotting paper and gently tapped with a glass rod to macerate root tips; and (v) the slide was covered with blotting paper and pressure was applied perpendicularly using the thumb, but avoiding lateral movement of the tissue, to spread the cells evenly. Stained preparations were observed under the microscope at 100x magnification. Ten fields showing active cell division were counted for determining the number of dividing / non-dividing cells. The mitotic index (MI) was calculated using data from 10 different squash preparations according to the formula $MI = \frac{\text{Number of dividing cells}}{\text{Total number of cells}} \times 100$.

2.6 Growth and development in the glasshouse

The growth and development of Sp5 and Otane were compared in a glass house experiment. Ten to fifteen seeds were grown in 25 cm x 10 cm plastic containers filled with commercially available potting soil mixed with slow release fertiliser. Only 10 healthy seedlings per pot were allowed to develop after one week. Pots were watered once every three days. Light and heat were provided by grow- lux

lights under automated control to provide suitable growing conditions. The date of planting is considered as Day 0. Regular measurements of plant height (base of the plant to the tip of the longest leaf) were made from Day 15 to 55.

2.7. Measurement of photosynthesis

The photosynthetic rate in 20 day old seedlings as well as the flag leaves of Otane, was compared with those of Sp5 using a portable open-path gas-exchange system Li-6400 (Li-Cor Inc., Lincoln, NE. USA). Photosynthetic rate measurements were carried out on glasshouse grown plants at ambient CO_2 level (300-400 μmol), light intensity (1500 μmol m^{-2} s^{-1}) and temperature (20˚C). Ten measurements were made and the average of these measurements was used to compare the rate of photosynthesis.

2.8. Effect of GA_3 on growth and development of wheat

The effects of GA_3 on the growth of Sp5, Otane (*Rht1*), CS971 (*Rht2*), Chuan-Mai 18 (*Rht8*), V Me S3 (*Rht12*), Rasp (*Rht13*), and Icaro (*Rht18*) were compared in a glass house experiment. Planting was carried out as described earlier. All plants were sprayed with an aqueous solution of 10 μM GA_3. The pH of the GA_3 solution was adjusted to 5.7 with 0.1N KOH solution and three drops of Tween20® per 100 ml solution was added as a wetting agent before spraying with an atomiser. GA_3 application continued until incipient run off. The control solution consisted of pure water and similar amounts of EtOH and Tween20®. Spraying was carried out on days 14, 21, 28 and 35 from sowing. The length between the base of the plant (soil surface) and the tip of the longest leaf was recorded as plant height.

2.9. Doubled haploid production

Doubled haploid production was carried out following the procedure of Campbell et al. (2000). 141 doubled haploids developed by Maqbool Ahmad at Crop & Food Research, Lincoln were also used in this study. Otane was crossed with Sp5 to generate hybrid F_1 seeds. The F_1 plants were used as the female parent and sweet corn (*Zea mays*) var. NZ71 as the male parent. Sweet corn and Otane were grown in pots in the glasshouse. Staggered planting (a week's gap) of corn seeds were done two to three weeks prior to planting of wheat, starting first week of September in order to match their flowering times.

Emasculation of wheat flowers was carried out 1-2 d before anthesis. Spikes with emasculated flowers were immediately bagged to prevent cross pollination and desiccation of injured floral parts. Emasculated flowers were visually observed for ripeness of the stigma from the second day after emasculation. Flowers with a receptive stigma (well developed with a cottony appearance) were pollinated with freshly gathered maize pollen. Old pollen was discarded by gently shaking the maize plant before fresh pollen was gathered by shaking the maize inflorescence into clean, dry paper boats. Pollen grains were introduced to the stigma using a fine, soft brush without damaging the stigma. Pollinated flowers (ears/ spikes) were immediately bagged to prevent cross contamination by pollen from other sources and possible desiccation. Pollinated spikes were sprayed with 100 mg/l 2,4-D solution one day after pollination. 2,4-D solution was prepared by dissolving it in a minimum amount of EtOH before diluting with pure water. 2,4-D solution was applied until the solution ran off the inflorescence.

Pollinated spikes were harvested after 14 days from pollination, washed in sterile water containing a few drops of Tween20® before isolating the fertilised ovaries (immature seeds) in a laminar flow hood. The well formed, plum-shaped diploid grains full of milky endosperm were separated and discarded. Haploid grains, which were not milky and plum- shaped were decontaminated with 20 percent (v/v) bleach (Chlorox®) for 3-5 minutes followed by several rinses with sterile water. Embryos were dissected out of the seeds under a microscope. Isolated embryos were cultured on modified MS (Murashige and Skoog, 1962) medium. The medium contained ½ strength macronutrients, 20 g l^{-1} sucrose and 12 g l^{-1} agar. Cultures were incubated in darkness at 22-24˚C until the embryos germinated. Germinated embryos were transferred to 16 h photoperiod (65µmol m^{-2} s^{-1}, PFD) provided by cool, white fluorescent lights. Haploid plants (3-5 cm) were washed to remove media sticking to their roots before transplanting to potting mix. These plants were kept under shade in the glasshouse for a week to acclimatise.

After three to four weeks in the glasshouse the haploid plants had typically produced 3-4 tillers. At this stage they were uprooted and washed to remove potting mix sticking on the roots. Shoots and roots of the plants were trimmed and minor incisions were made at the base of their stem to permit greater absorption of colchicine. The plants were kept in 500 mg l^{-1} colchicine solution prepared in 10%

(v/v) dimethyl sulfoximide (DMSO) for six hours at 30˚C. The colchicine solution was stirred continuously to keep it aerated. The treated plants were washed with cold tap water for five minutes before re-potting. Potted plants were maintained in the glasshouse for further development. Pre-existing shoots died off at this stage and new shoots developed. These plants were grown to maturity and seeds were harvested at maturity.

2.10. Effect of GA_3 on the doubled haploid population

The effect of GA_3 on the doubled haploids was determined in a glasshouse experiment. The doubled haploids were grown in a split plot design (Table 2.1) model. GA_3 treatment was applied as described in Section 2.8. The trial was a 140 x 2 factorial structure with 140 wheat lines and two GA_3 treatments (GA_3 & Control). Due to the high number of treatments (280) there were only two replicates leading to a total of 560 plots. Each plot was a pot with 6 wheat plants. There were two Blocks, and two Tables (Main-Plots) within each block. The GA_3 treatment was randomly applied to one whole table within each block, leading to a Split-plot design. Each table had 140 sub-plots (Pots), with the Wheat factor arranged in a random Row-Column (10x14) Design on four replicate tables. This design provided protection against any possible confounding caused by trends that may run across the rows or the columns in the 10x14 grid. Due to the split-plot nature of the design, the GA_3 treatment was weak with only four observations with two replicates for each treatment. However the wheat variety and interaction terms were well replicated.

A baseline measurement of plant height was recorded 10 days after sowing, then, the treatment was applied. Repeated measurements of plant height were recorded weekly following the treatment. Analysis was based on the total growth (soil surface to the tip of the ear) at the end of the trial since treatment application.

Statistical Analysis

The data had a split-plot design, repeated measures, and a covariate in the form of the baseline growth measurements after 10 days. The covariate was incorporated into the analysis by subtracting baseline growth from each subsequent height measurement, leading to a 'Final Growth' variate. Final growth at the last time was analysed using Analysis of Variance in GenStat v. 8.

The data followed the assumptions of normality and equal variance required to carry out ANOVA. There were some outliers (~ 10), but this was to be expected with a sample size this large (n = 560). An indication of the variability associated with means is given by the Least Significant Difference (LSD). The LSD is the smallest difference needed between two means for the means to be considered statistically significantly different. The LSD is based on a probability of 0.05. Plots presenting treatment and sampling date means with appropriate LSD's were produced in SigmaPlot v.9.

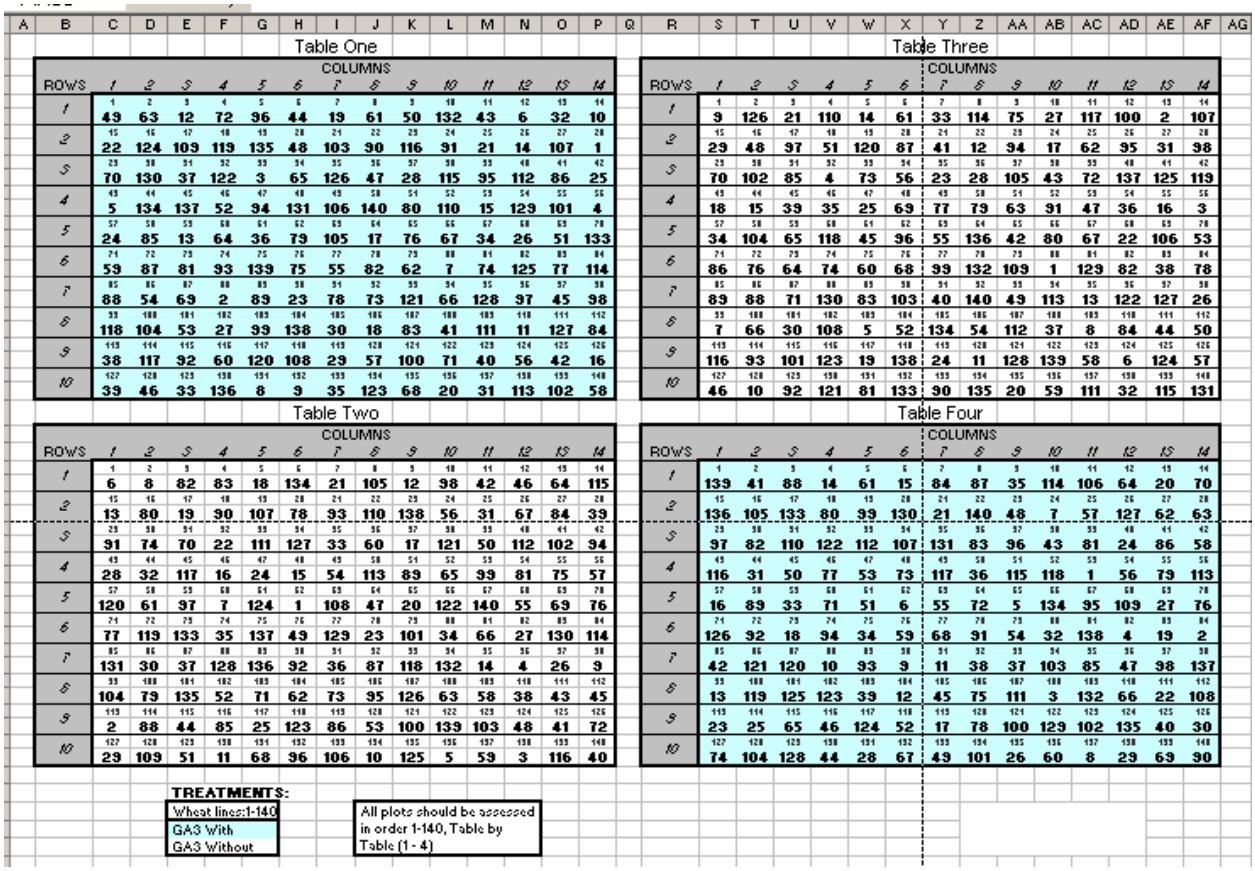


Fig. 2.1 Split plot design used to study the effect of GA_3 on the doubled haploid population

2.11. Grain measurements

Individual grains were measured for their length and width at the widest point using a digital Vernier calliper. The length and width of the embryo was also measured similarly. Following these measurements, the grain was cut in half across its width. Crease measurements (depth and width) were determined as shown in Fig 2.1. Parameters such as plant height, ear length and length of the awns were determined

using a graduated scale. Thousand seed weight was determined using a microbalance (Sartorius®, Germany). In all cases 20 measurements were used to reach the average values.

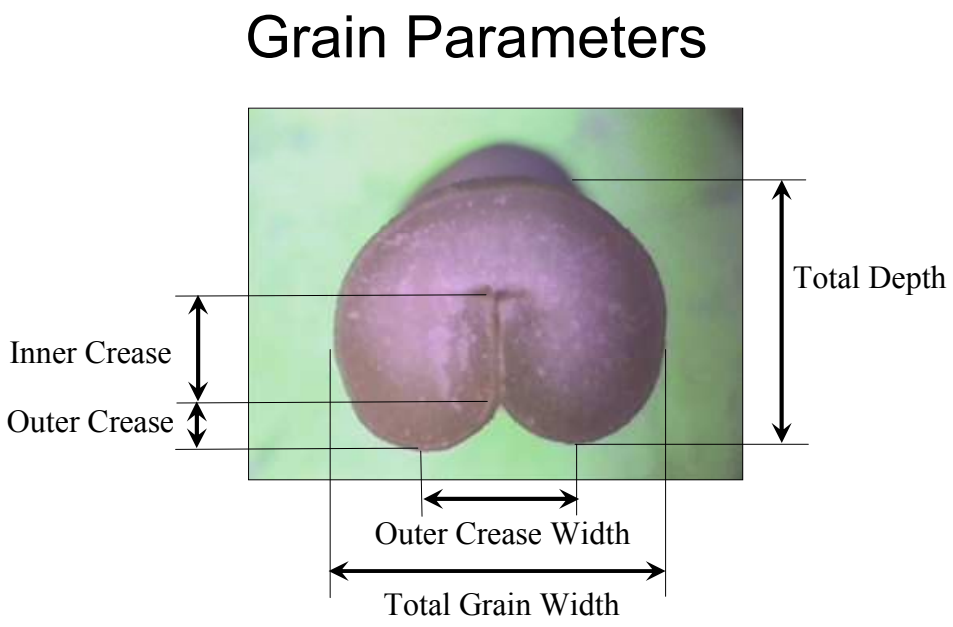


Fig.2.2. Graphic representation of grain parameter measurements

2.12. Mutation studies

Otane and Sp5 seeds were subjected to different levels of gamma radiation. Four seed lots of approximately 20,000 seeds were subjected to 100, 150 Rad, 200 and 250 gray gamma radiation at the National Radiation Laboratory (NRL), Christchurch. The irradiated seeds **(**25 grains/ treatment**)** were test planted in pots to evaluate the effect of radiation on their germination and establishment. From this analysis, rates of 100 and 150 gray were chosen for further study. One kilogram of seed lots of Otane and Sp5 were then irradiated at each dose and field sown at the experimental field of Crop& Food Research, Lincoln in September 2005.

Once the plants from gamma irradiated seeds were nearly mature, the plots were scanned for chimeric plants. Plants in the Sp5 plots showing Otane type spike(s) along with Sp5 type spike(s) on the same plant were recorded as chimeric plants (Fig. 2.4). Individual plants showing this chimerism were uprooted for threshing. Extreme care was taken to avoid any possible mistakes whereby two closely positioned plants were recorded as one chimeric plant. Ears were gathered from individual chimeric

plants and threshed separately. They were labelled as mutant lines and stored to create the next generation. According to convention the treated seed was labelled M0 seed and the plants that grew from it were M0 plants. The seed gathered from their heads constituted the M1 seed, and the plants which grew from that formed the M1 population.

The M_1 population was raised by planting 12 seeds per pot in the environment regulated (23 ± 3˚C, about 100 µmol m^{-2} s^{-1} PFD) glass house at the Crop& Food Research, Lincoln during May 2006. These plants were screened for the segregation of traits characteristic of the shaerococcum phenotype. Plants from pots showing little segregation were harvested, threshed individually and planted to generate the M_2 generation. The M_2 generation was raised in field plots in October 2006 as the climate suited the production schedule in the field. Mutant plants were again gathered and processed as described above to generate the M_3 population.

2.13. Genetic mapping

One hundred and fifty doubled haploids along with their parents (Otane and Sp5) were scanned with microsatellite (simple sequence repeat - SSR) markers. Primer details of these SSR markers of wheat were gathered from the literature (Roder et al. 1998; Somers et al. 2004). Detailed information available on the United States Department of Agriculture (USDA) website http://wheat.pw.usda.gov/GG2/index.shtml) was also used as reference for this work. In addition, 134 doubled haploids, selected to reduce a possible bias in the genetic analysis towards Otane characteristics along with the parents (Otane and Sp5) were subjected to diversity arrays technology (DArT) profiling through the service provided by Triticarte Pvt. Ltd. Australia. Diversity arrays technology is a cost effective microarray-hybridisation-based technique that enables the simultaneous typing of several hundred polymorphic loci spread over the genome without the need for prior sequence information (Jaccoud et al. 2001; Wenzel et al. 2004).

Microsatellite marker analysis and construction of genetic map

Total genomic DNA was extracted from young, healthy leaves of 10-15 day old seedlings (individual plants) of the 150 doubled haploids and parents (Otane and Sp5) using DNeasy plant kit (Qiagen, Australia Pty. Ltd). Microsatellite polymorphism was determined using parental DNA. Primer sequences of gwm, gdm,

wmc, and barc microsatellite markers used in the study were available from Roder et al. (1998); Pestsova et al. (2000); Somers et al. (2004) and the Graingenes website (http://wheat.pw.usda.gov/GG2/index.shtml).

The polymerase chain reaction (PCR) and data collection

The polymerase chain reaction (PCR) used for determining microsatellite polymorphism was carried out according to Roder et al. (1998) or modified according to Schuelke (2000). The microsatellite analysis procedure of Scheulke (2000) required the use of M13 tailed (M13 sequence CACGACGTTGTAAAACGAC) forward primers. These primers were ordered from Invitrogen, USA. Typically, a PCR mix included 10x buffer, dNTPs, fluorochromes ABI- FAM (6-carboxy-fluorescine) or HEX (hexachloro-6-carboxy-fluorescine, forward and reverse pimers, Taq polymerase, genomic DNA and water to make up 15 µl, as shown in Table 2.2.

Table 2.2 Details of a typical PCR mix used in this study

Components of the reaction mix	Quantity
10 x buffer + Mg++	1.5 µl
10 mM dNTPs	0.24 µl
20 µM ABI-FAM (1/4)	0.15 µl
5 µM:20 µM Forward :Reverse primer	2.4 µl
H_2O	7.59 µl
Taq polymerase	0.12 µl
Genomic DNA	3.0 µl
Reaction volume	15.0 µl

Reactions were multiplexed using fluorescent labelled (FAM/ HEX) primers.

Different temperature settings were used for the PCR amplification of primer sets according to published information (Roder et al. 1998; Pestova et al. 2000; Somers et al. 2004) or the Graingenes website (http://wheat.pw.usda.gov/GG2/index.shtml). PCR conditions for a primer with a Tm of 60˚C included touchdown 62˚C to 58˚C over 4 cycles, then 36 cycles at 58˚C, followed by a final extension of 45 minutes at 72˚C. PCR reactions were carried out in an Eppendorf Mastercycler ep 384.

Microsatellite analysis was carried out following a standard operating procedure used at Crop & Food Research Ltd., Lincoln, which is based on Scheulke

(2000). The PCR product (1.5ul) was precipitated using 2 µl of freshly prepared precipitation mix (11 ml 95% (v/v) EtOH + 0.9 ml 3M NaOAc, pH 4.8) in an ABI PCR plate. The plate was sealed with aluminium seal and inverted to mix. The plate was spun at 3200G for 30 min. in an Eppendorf 5810R centrifuge for sedimentation. Immediately after the spin the seal was removed and the plate was inverted onto tissue paper and spun at 200G. The plate was dried at 37˚C for 10 min. in an incubator. The sedimented product was resuspended in 10 µl formamide (Hi-Di) + 0.075 µl ROX 400 (6 carboxy- rhodamine) ladder, spun down (200G) and denatured at 95˚C for 5 min., then cooled to 4˚C on ice for 5 min. Raw data of microsatellite fragments were collected using fluorescent capillary electrophoresis on an ABI 3100 genetic analyser (Applied Biosystems, Foster City, CA, USA). Data were analysed using GeneMapper v3.7 (Applied Biosystems, USA).

2.14. Map construction and QTL analysis

A composite map using the SSR markers and DArT markers was generated using Mapmanager QTX 3.7 (Manly et al. 2001) downloaded from (www.mapmanager.org/mmQTX.html). QTL analysis was also carried out with Mapmanager QTX 3.7 using SSR markers, DArT markers, characters of grains (Appendix II) and plants (Appendix III) including grain width, grain length, crease size, embryo size, plant height and ear length.

2.15. Statistical considerations

Experiments were designed and results were analysed with the help of statisticians at Crop & Food Research, Lincoln. Graphs and tables were generated using Microsoft Excel or statistical software Genestat®, SAS or Sigmaplot.

CHAPTER THREE

RESULTS

3.1. Comparison of gross morphology, growth and development of *T. sphaerococcum* var. Sp5 and *T. aestivum* cv. Otane.

3.1.1. Morphology

T. sphaerococcum (Sp5) is characterised by a semidwarf phenotype with short, strong culms, short and compact ears, short awns, hemispherical glumes and round grains compared to *T. aestivum* (Fig 3.1).

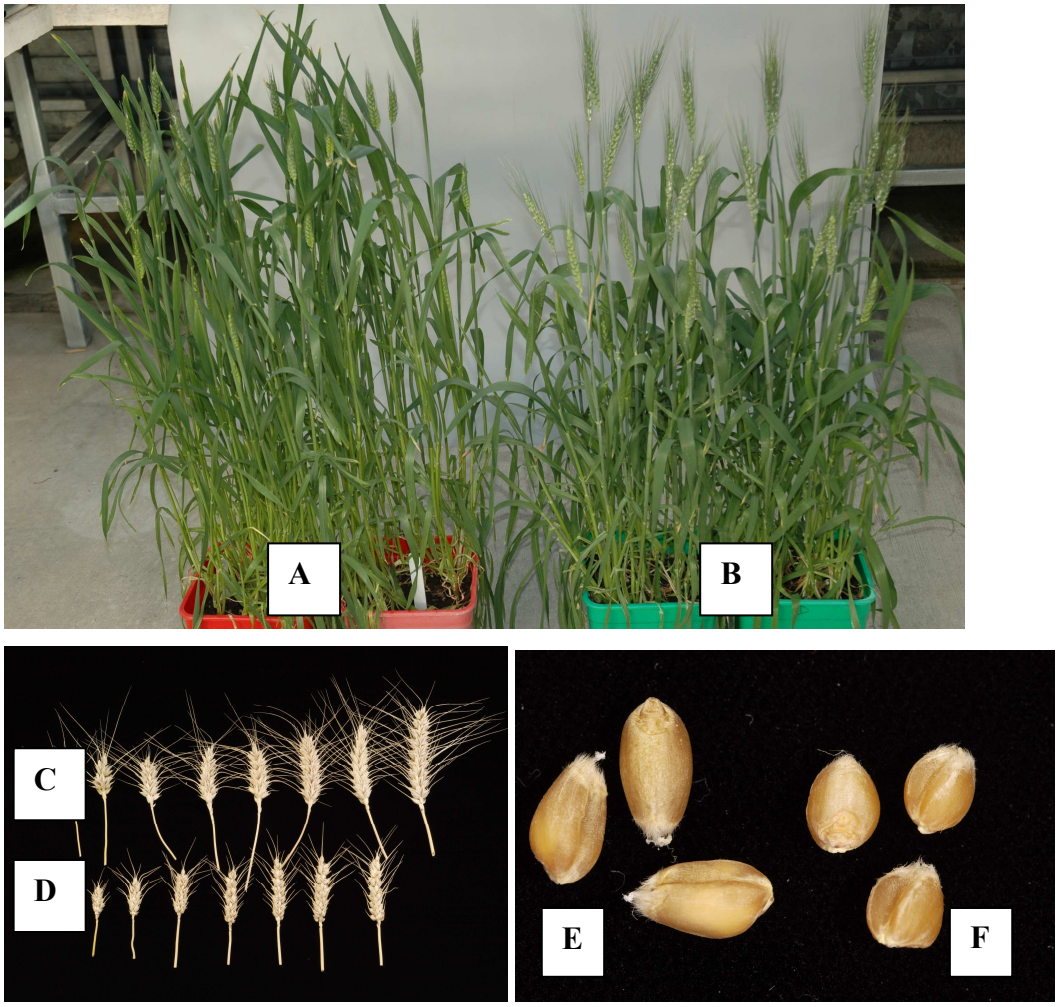


Fig 3.1. Comparative morphology of *T. sphaerococcum* (Sp5) and *T. aestivum* (Otane) varieties. (A) Whole plant - Sp5, (B) Whole plant – Otane, (C) Spikes of Otane, (D) Spikes of Sp5, (E) Grains of Otane and (F) Grains of Sp5.

3.1.2. Seed germination

Sp5 and Otane seeds, stored for a year at room temperature showed similar rates of germination (90-100%) in a laboratory study (Fig. 3.2).

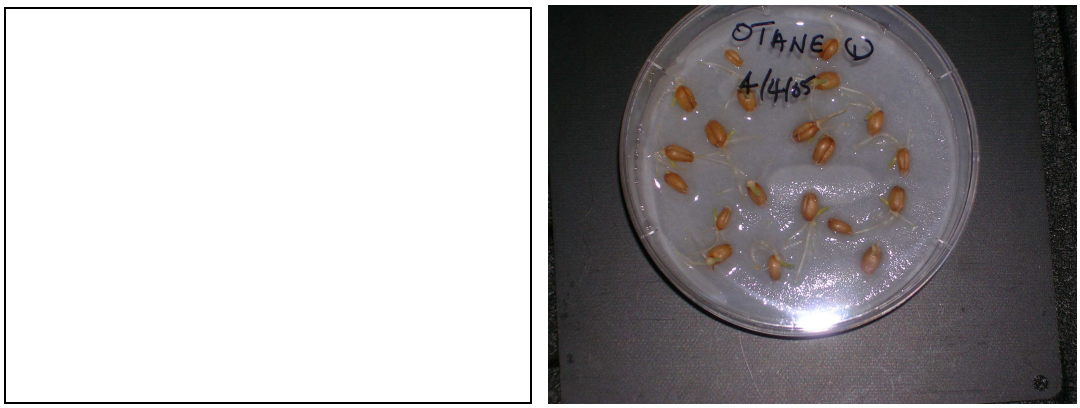


Fig 3.2 Germinating seeds of Sp5 (left) and Otane (right)

3.1.3. Comparison of early seedling growth

Early seedling growth of Otane and Sp5 was compared *in vitro* (Fig 3.3) and *in vivo* (Fig 3.4). Otane seedlings grew faster than Sp5 seedlings in the first week (Table 3.1).

Fig 3.3 Comparison of three day old seedlings grown *in vitro* (A) Sp5 (B) Otane

Table 3.1 Shoot and root lengths of in vitro grown Otane and Sp5 seedlings measured on 3rd and 4th day from incubation. Data presented is the average of 16 measurements ± SE (LSD = 6).

	Shoot length (mm)		Root length (mm)	
	Otane	Sp5	Otane	Sp5
Day 3	27.1 ± 4.9	17.5 ± 3.9	30.5 ± 5.3	25.1 ± 5.7
Day 4	39.2 ± 5.4	29.5 ± 4.1	76.2 ± 8.8	50.0 ± 7.7

Shoot and root growth of Otane and Sp5 were compared on day 3 and 4 from incubation. Otane had significantly greater growth compared to Sp5 three and four days after incubation.

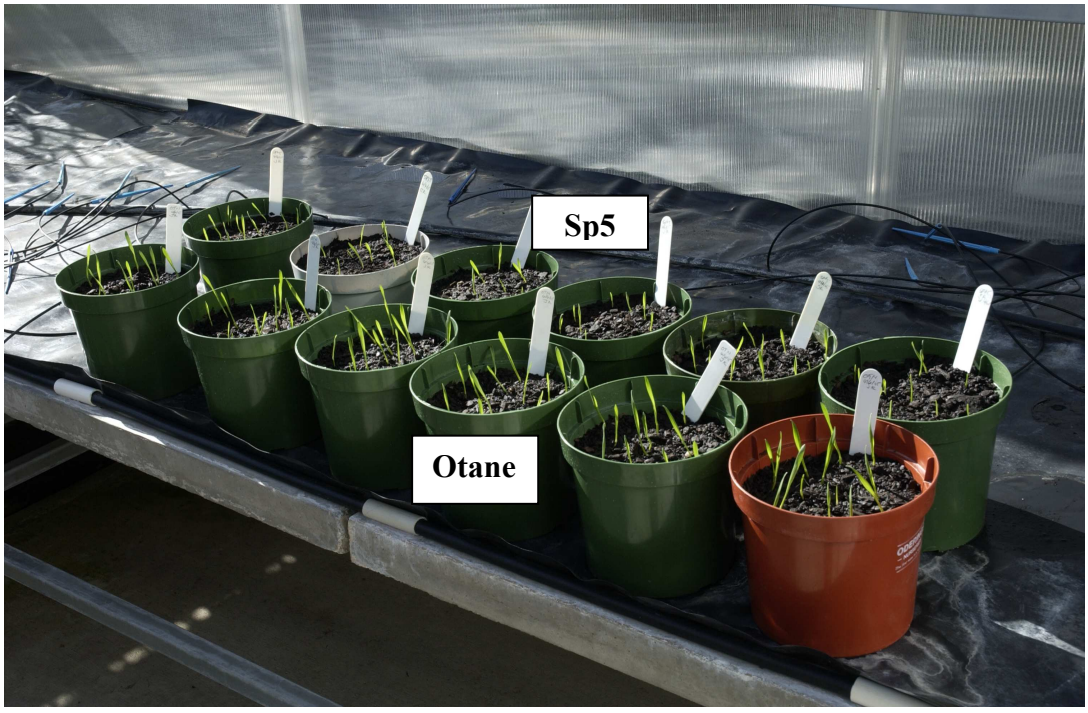


Fig 3.4 Comparative growth *in vivo* of Otane (row 1) and Sp5 (row 2) by day 8 from sowing.

3.1.4. Mitotic index

The mitotic index of root tip cells of three day old seedlings of Sp5 and Otane was determined using squash preparations (Fig 3.5). Otane had a higher mitotic index (6.9%) compared to the Sp5 (5.8%).

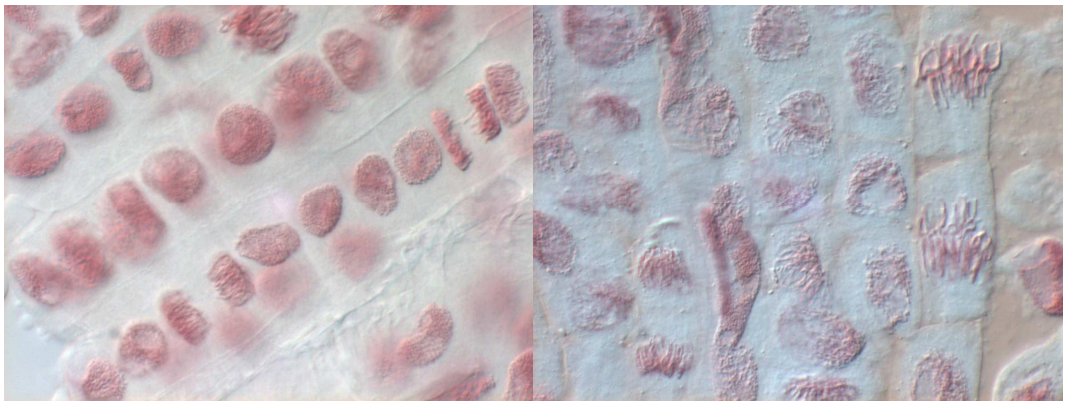


Fig 3.5 Squash preparations of Sp5 (left) and Otane (right) root tips (x1000)

3.1.5 Further growth and development

Sp5 seedlings attained comparable growth in height with Otane by day15 from sowing. Otane reached anthesis by day 50 from planting while Sp5 was only heading by that time (Fig 3.6). Both varieties matured in 135 days (Fig 3.7).

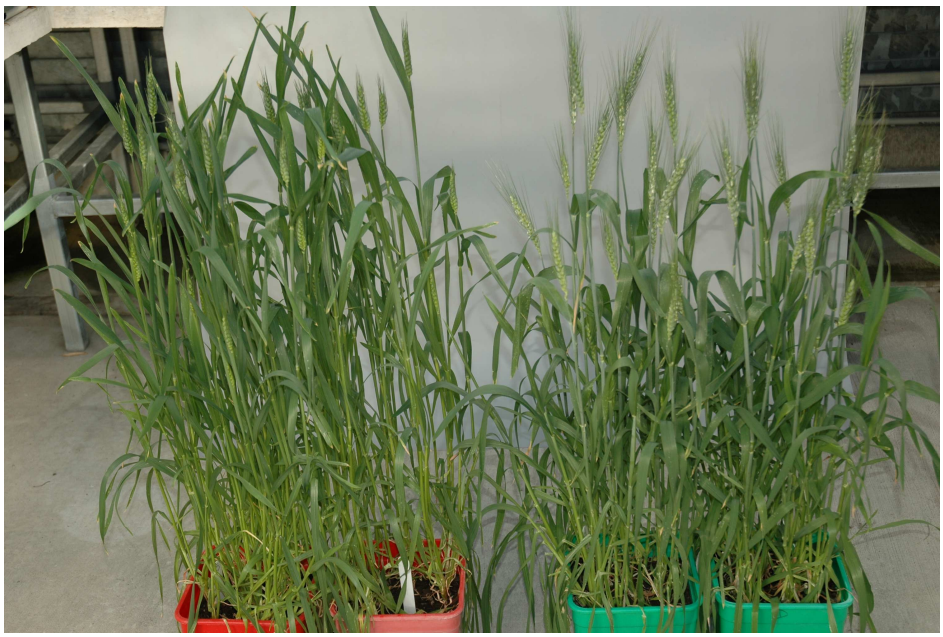


Fig 3.6 Comparative development of Sp5 (left) and Otane (right) by day 50 from planting. Otane progressed to anthesis while Sp5 remained in the heading stage.

Fig.3.7. Otane and Sp5 matured about the same time (day 135), (Ot-left, Sp5–right).

3.1.6. Photosynthetic rate

Otane had thicker and darker green leaves compared to the Sp5. Despite the apparent difference in leaf morphology, they recorded similar photosynthetic rates (Table 3.1).

Table 3.1 Comparative rates of photosynthesis in Otane and Sp5, 20 days and 50 days from sowing. The data presented is average ± SE of ten measurements (LSD = 4.2).

Rates of photosynthesis ($\mu mol\ s^{-1}\ m^{-2}$) ± SE			
Seedlings (day 20)		Flag leaf (day 50)	
Otane	Sp5	Otane	Sp5
21.24 ±1.09	18.42 ±1.78	22.59 ±1.43	20.28 ±1.54

Photosynthetic rates were determined on day 20 (seedling stage leaf) and day 50 (flag leaf). The photosynthetic rates were not significantly different between the varieties.

3.2. Effect of plant hormones on growth and development of wheat

3.2.1. Effect of growth hormones on seedling growth in vitro

GA_3 enhanced shoot growth *in vitro* of both varieties at low concentrations (0.1 -10 mg l^{-1}) but reduced growth at 100 mg l^{-1} ($P<0.01$), (Fig 3.8 and 3.9). Although lower concentrations of IBA did not significantly reduce shoot growth, 100 mg l^{-1} significantly reduced shoot growth in both varieties by 96 hours from incubation ($P<0.01$), (Fig 3.10). BAP significantly reduced shoot growth of both varieties *in*

vitro at 100 µM concentration by 144 hours from the start of incubation (P<0.01), (Fig.3.11).

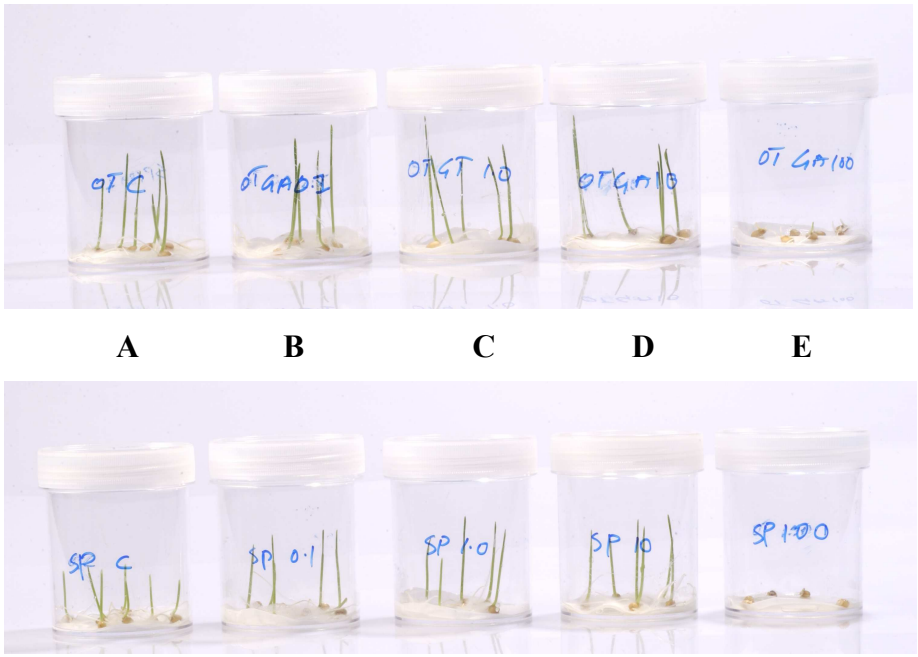


Fig 3.8 Effect of GA_3 on the growth of wheat seedlings *in vitro*: photographed on day 5 from incubation. (A) 0.0 mg l^{-1} (B) 0.1 mg l^{-1} (C) 1.0 mg l^{-1} (D) 10.0 mg l^{-1} (E) 100 mg l^{-1} GA_3.

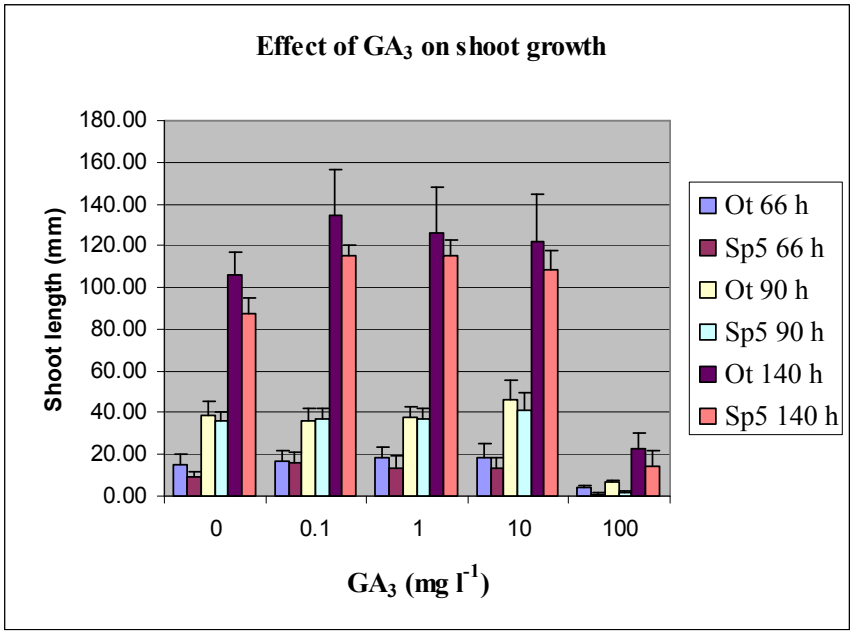


Fig 3.9 Effect of GA_3 on shoot growth *in vitro* of Otane and Sp5 seedlings. Error bars indicate standard error (SE).

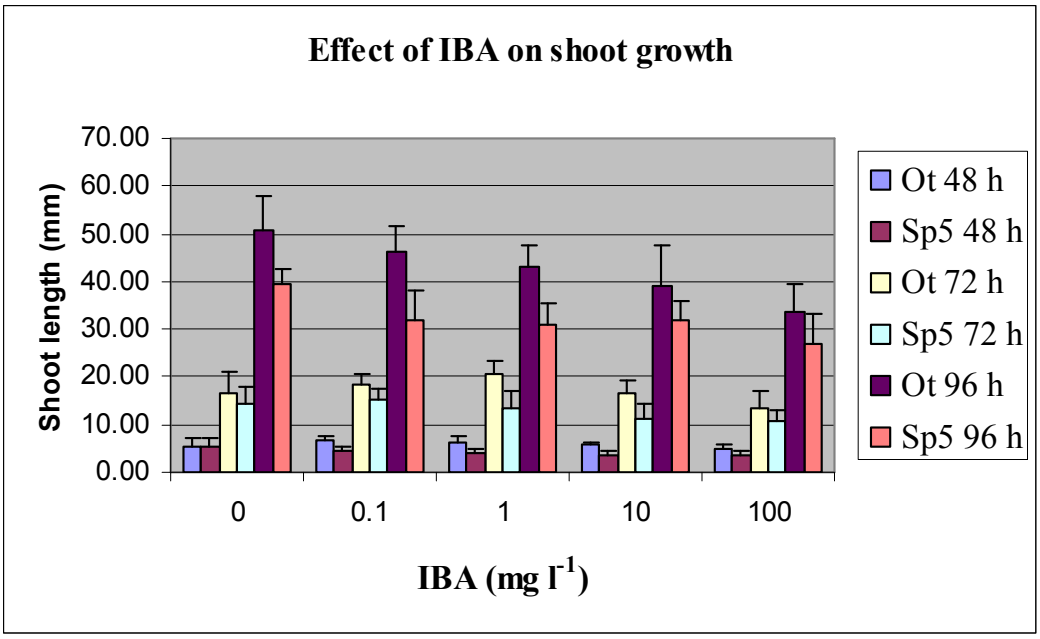


Fig 3.10 Effect of IBA on shoot growth *in vitro* of Otane and Sp5 seedlings. Error bars indicate SE.

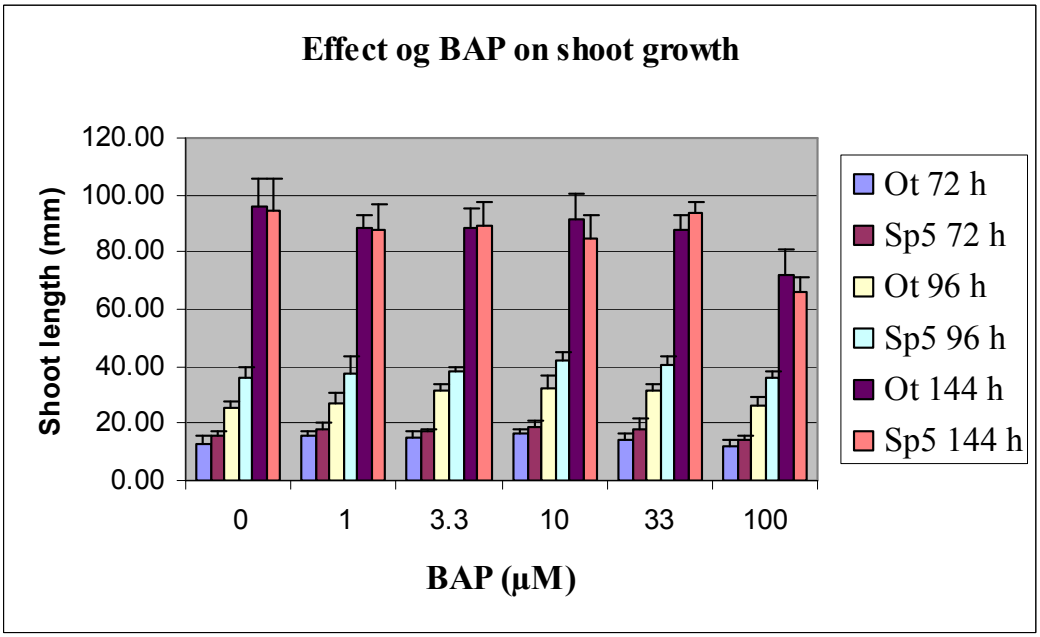


Fig 3.11 Effect of BAP on shoot growth *in vitro* of Otane and Sp5 seedlings. Error bars indicate SE

Table 3.3 ANOVA of effects of different factors on early growth of Sp5 and Otane wheat seedlings.

Facrors	GA_3	IBA	BAP
Variety	P<0.01	P<0. 01	P<0.01
Time	P<0.01	P<0. 01	P<0.01
Concentration	P<0.01	P<0. 01	P<0.01
Variety*Time	P<0.01	P<0. 01	P<0.01
Time*Concentration	P<0.01	P<0. 01	P<0.01
Variety*Concentration	P 0.84	P 0.24	P 0.53
Variety*Time*Concentration	P 0.88	P 0.49	P 0.60

Seedlings of Sp5 and Otane varieties showed a similar trend in their response to the applied GA3, IBA and BAP (Table 3.3)

3.2.2. Effects of GA_3 on growth of wheat in vivo

A single application of 10 mg l^{-1} GA_3 appeared to cause a transient increase in the growth of Sp5 (Fig 3.12) but did not enhance the growth of Otane.

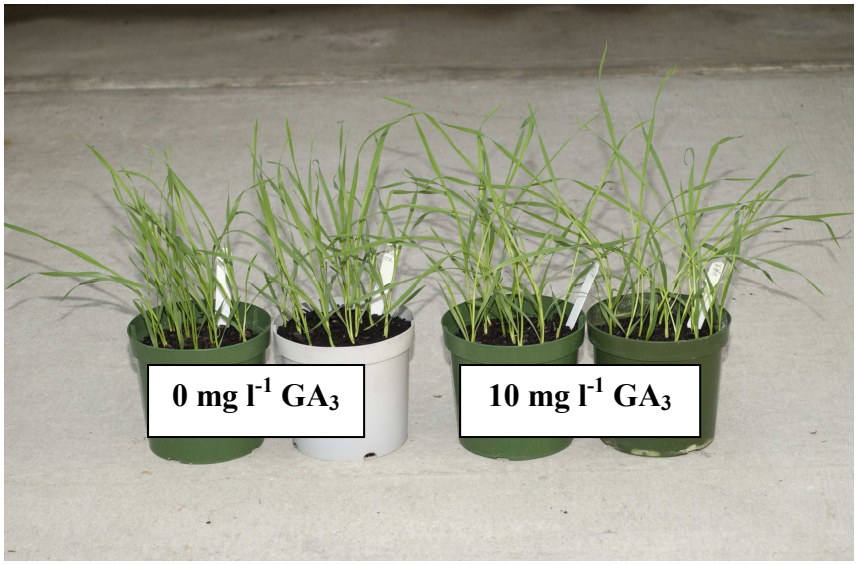


Fig 3.12 Effect of one foliar application of 10 mg l^{-1} GA_3 on Sp5 seedlings. Photographed on day 21 following the GA_3 application on day 16 from planting.

3.2.3. Comparison of the effect of GA_3 on Sp5 and **T. aestivum** *cultivars with a known Rht background.*

In another glasshouse experiment, *T. sphaerococcum* (Sp5) was compared to six *T. aestivum* cultivars with a known *Rht* background for their response to GA_3. The cultivars included in the experiment were Otane (*Rht1*) and CS 971 (*Rht2*) both of which are unresponsive to GA_3, and Chun-Mai 18 (*Rht8*), VMeS3 (*Rht12*), Rasp (*Rht13*), and Icaro (*Rht18*) which are responsive to GA_3 (Pers. Commun. Dr.

Rebetzke, CSIRO, Australia). One group of these plants was sprayed with aqueous GA_3 (10 mgl^{-1}) and the control received no GA_3 application.

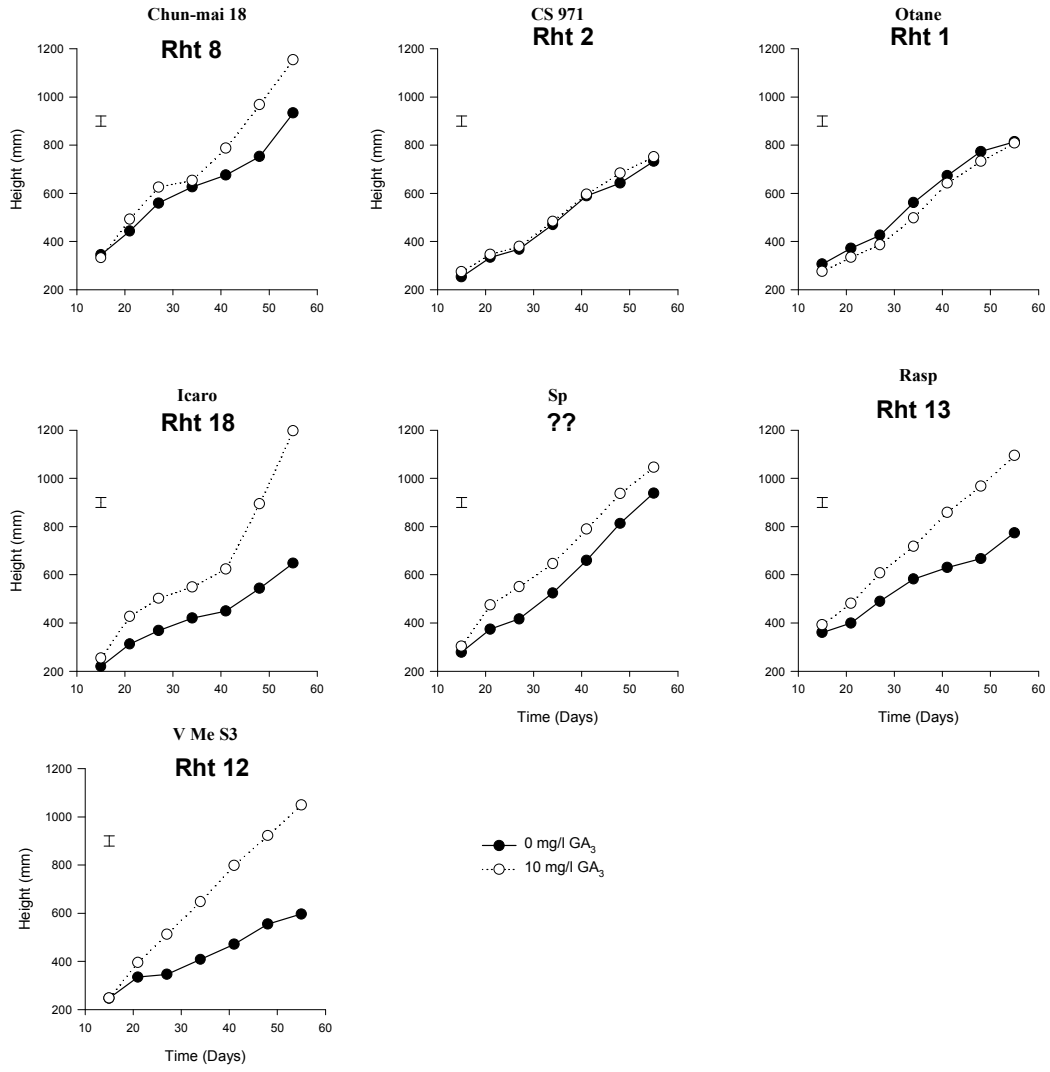


Fig 3.13 Growth patterns of Sp5 and different *Rht* wheat varieties in response to 10 mg l^{-1} GA_3 application (LSD 5%). The plants in the treatment group were sprayed with 10 mg l^{-1} GA_3 on weekly intervals starting day 14 from sowing. Plant height (soil surface to the tip of the longest leaf or to the tip of the fully developed ear on day 60) was measured at dates specified in the graph.

Their growth and development were monitored until day 60, by which time they had all set seeds. Results of this study showed that the growth pattern of Sp5 differed from both the GA_3 unresponsive genotypes and the GA_3 responsive genotypes of *T. aestivum* varieties included in this study. Sp5 remained responsive to

the applied GA_3 in its early developmental stage (day 15 – day 30) but the applied GA_3 had no significant influence after 30 days of growth (Fig. 3.13).

Growth of varieties Otane (*Rht 1*) and CS 971 (*Rht 2*) was not influenced by the application of GA_3. Var. Chun-Mai 18 (*Rht 8*) showed an increase in growth following first application of GA_3 and the growth rate dramatically increased from day 35. Varieties V Me S3 (*Rht 12*) and Rasp (Rht 13) recorded a steady increase in growth with the first application of GA_3 and the higher rate of growth was maintained through the entire observation period. Variety Icaro (*Rht 18*) treated with GA_3 showed a continuous growth increase from day 15. This variety also established a large growth difference starting day 35 between the treatment and control. The control group of taller varieties (Chum Mai18, Icaro and Rasp) recorded higher growth rates from day 30-35 unlike the shorter varieties (Otane, CS 971, VMe S3 and Sp5), which showed higher growth rate from day 25.

3.2.4. Response of the doubled haploid population to GA_3.

The doubled haploid population used for gene mapping was subjected to a similar GA_3 treatment (Section 2.10).

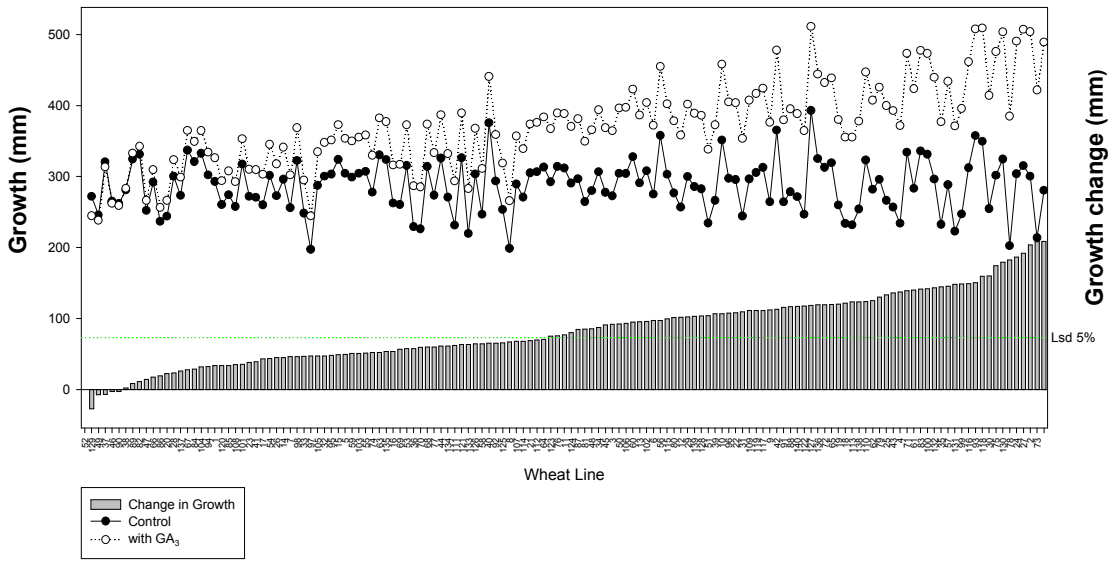


Fig 3.14 Effect of 10 mg l^{-1} GA_3 on the growth and growth change of the doubled haploid population (LSD 5% for 56 df). Growth change is the difference in final plant height (soil surface to the tip of the ear) between control and GA_3 treated plants on day 60.

Individuals in the doubled haploid population showed considerable variation in their response to the applied GA_3 (Fig 3.14). Appendix I provides a readable list of the doubled haploids used to compile Fig. 3.14.

3.3. Phenotypic characterisation of the doubled haploids

The doubled haploid population was characterised for grain parameters {grain shape, grain length, grain width, grain depth, grain length /width, outer crease, inner crease, embryo length, embryo width, and embryo length /width (Appendix II)} and plant form {plant height, spike length and awn length (Appendix III)}. A multivariate analysis performed on these characteristics indicated that the whole population segregated into a *T. sphaerococcum* group (Sp5) and a *T. aestivum* group (Ot). This segregation into two distinct groups indicated a tight linkage of the sphaerococcum complex (Fig. 3.15). Multivariate analysis of whole plant characteristics such as mean plant height, mean awn length and mean head height (Fig 3.15a) showed a looser association of individuals within the *T. sphaerococcum* group and *T. aestivum* group compared to the multivariate analysis for grain parameters such as grain length / width, grain length / depth and % inner crease along with one of the whole plant traits such as mean plant height, mean head height or mean awn length (Fig 3.15b, 3.15c and 3.15d).

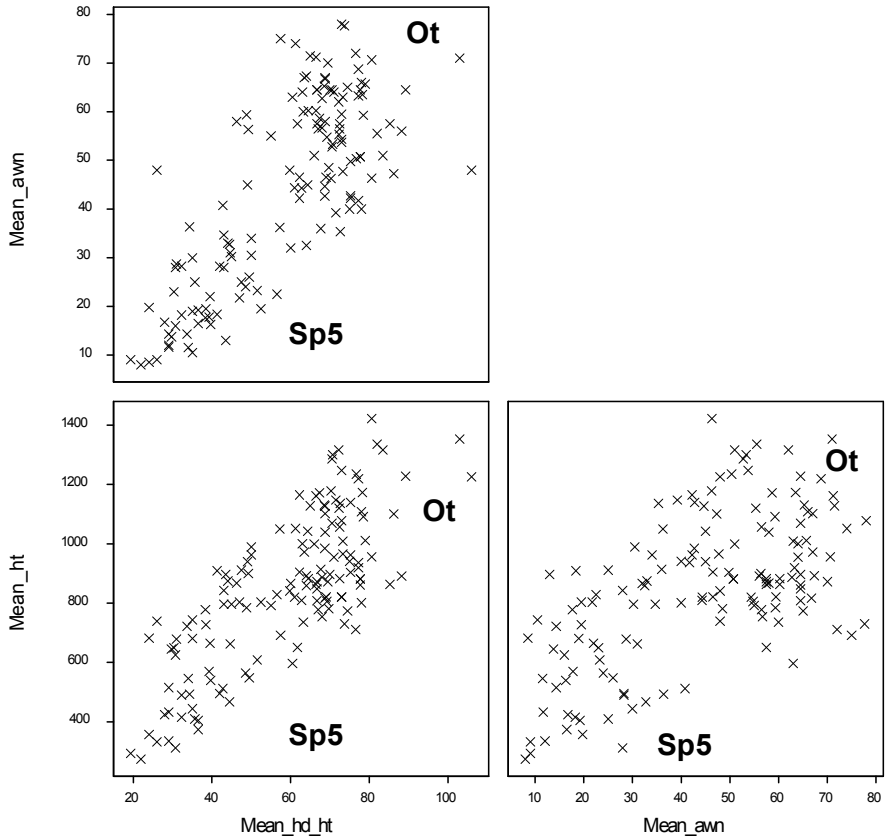


Fig 3.15a Multivariate analysis for gross morphological characters (plant height (Mean_ht), awn length (Mean_awn) and head height (Mean_hd_ht)) of the doubled haploid population.

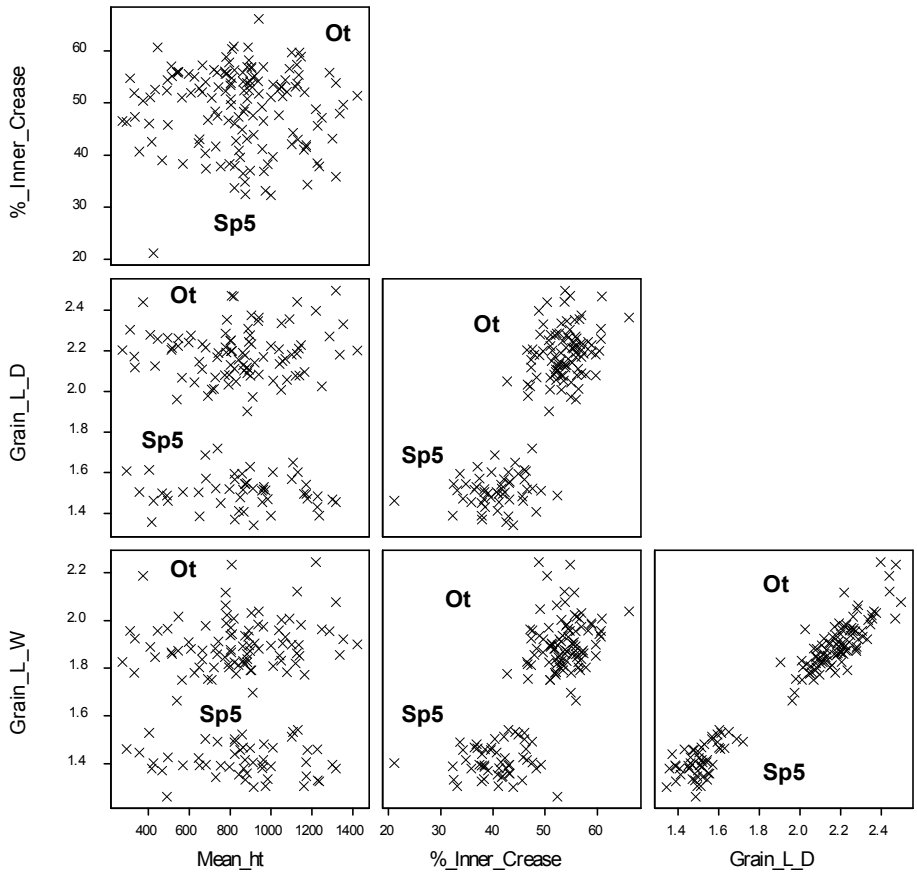


Fig 3.15b Multivariate analysis for grain parameters (grain length / width (Grain_L_W), grain length / depth (Grain_L_D), % inner crease (%_inner_crease) and plant height (Mean_ht) of the doubled haploid population.

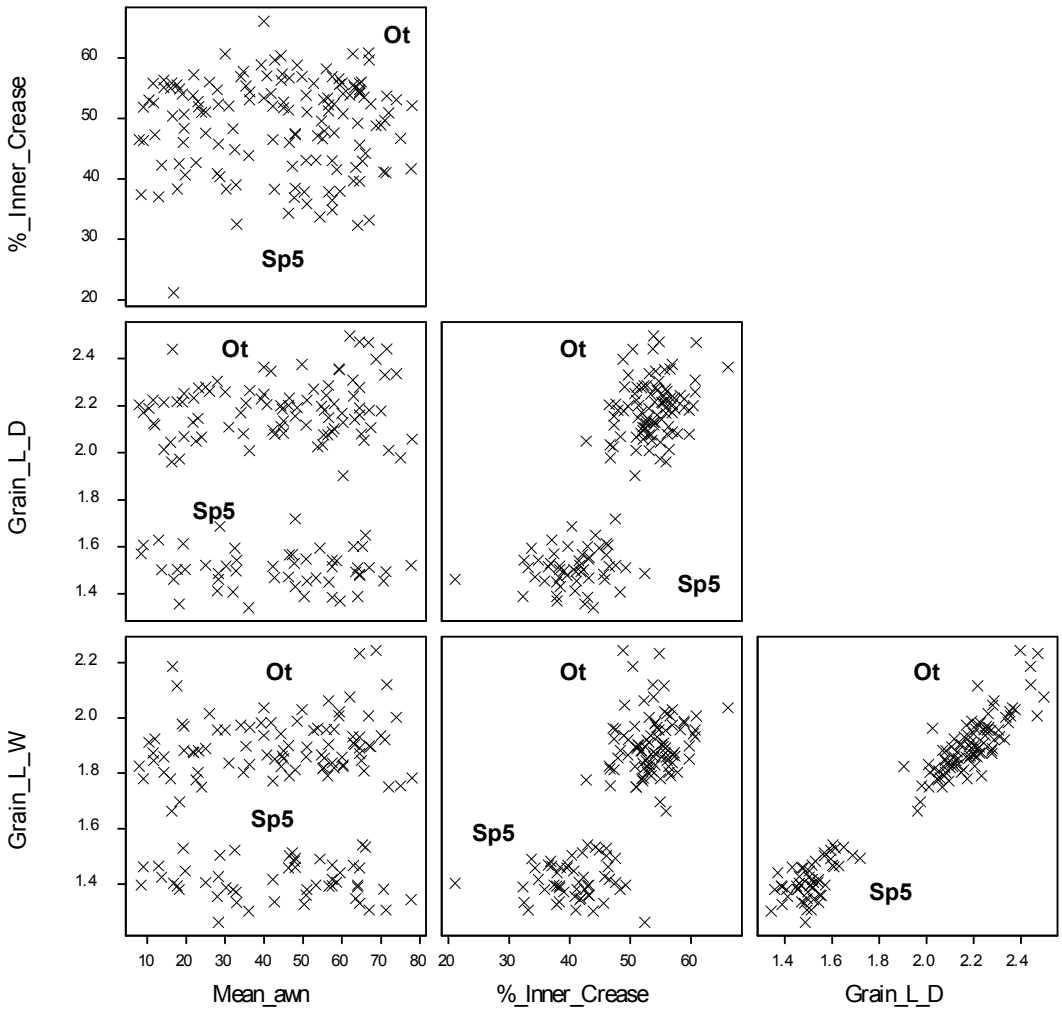


Fig 3.15c Multivariate analysis for grain parameters (Grain length / width (Grain_L_W), Grain length / depth (Grain_L_D), % Inner crease (%_Inner_crease) and mean Awn length (Mean_awn)) of the doubled haploid population.

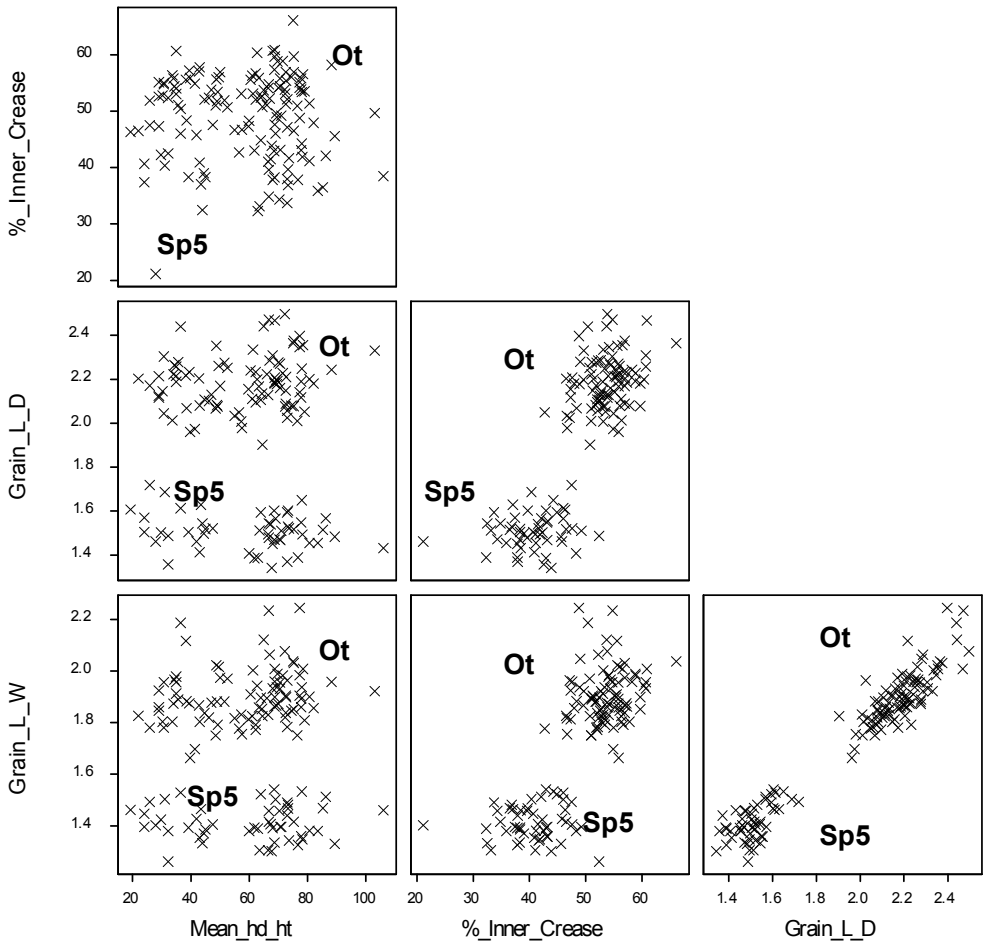


Fig 3.15d Multivariate analysis for grain parameters (grain length / width (Grain_L_W), Grain length / depth (Grain_L_D), % Inner crease (% Inner_crease) and Head height (Mean_hd_ht)) of the doubled haploid population.

3.4. Molecular marker analysis and gene mapping

The genomes of the parental varieties, Otane and Sp5, were scanned with 348 microsatellite markers. Only 169 of the 348 markers or 49% of the markers (Table 3.4) were polymorphic between the parents.

Table 3.4 Microsatellite markers that are polymorphic between Otane and Sp5 varieties

Marker ID	Chrom. #	Marker ID	Chrom. #	Marker ID	Chrom. #
cfa2219	1A	wmc656	3D	gwn644	6B
barc158	1A	CFD9	3D	gwm193	6B
wms136	1A	WMS3	3D	gmw88	6B
wmc24	1A	gdm72	3D	gmw626	6B
wms33	1A	barc71	3D	wmc486	6B
barc119	1A	gwm52	3D	wmc494	6B
gwm164	1A	gwm383	3D	barc178	6B
wm357	1A	gwm610	4A	barc134	6B
gwm99	1A	gwm397	4A	wmc494	6B
wmc254	1A,4B,6A	gwm160	4A	barc134	6B
wmc59	1A,6B	wmc219	4A	barc178	6B
gdm33	1A,1D	wmc258	4A,5B	barc178	6B
gwm550	1B	wmc283	4A,7A	arc134	6B
gwm582	1B	barc70	4A, 7A,D	barc175	6D
gwm273	1B	gwm149	4B	barc96	6D
gwm413	1B	gwm251	4B	gwm469	6D
gwm403	1B	gwm6	4B	gwm325	6D
gwm153	1B	gwm194	4D	cfd49	6D
gwm337	1D	barc151	5A, 7A	cfd75	6D
wmc429	1D	wmc475	5A	cfd76	6D
wmc36	1D	wmc727	5A	gwm666	7A
cfd63	1D	barc151	5A/7A	gwm471	7A
gwm232	1D	wmc727	5A	gwm573	7A
gwm106	1D	barc94	5A, 7B	gwm276	7A
barc66	1D,2B,7D	gwm443	5A, 5B	gwm63	7A
cfd19	1D,5D,6D	wmc475	5A,7B	gwm332	7A
gwm636	2A	gwm304	5A	wmc479	7A
gwm359	2A	gwm154	5A	wmc168	7A
gwm294	2A	gwm186	5A	wmc83	7A
gwm356	2A	gwm666	5A	barc108	7A
wmc154	2B	gwm126	5A	barc154	7A,D
gwm429	2B	gwm595	5A	cfa2040	7A,B,D
gwm148	2B	gwm234	5B	barc121	7A,D
gwm526	2B	gwm443	5B	wmc517	7B
gwm410	2B,5A	gwm540	5B	gwm569	7B
barc142	2D, 5A, 5B, 6A	gwm371	5B	gwm537	7B
gwm455	2D	gwm499	5B	gwm400	7B
gwm484	2D	gwm408	5B	wm46	7B
gwm102	2D	gwm604	5B	gwm16	7B
gwm301	2D	cfd5	5B/6D	gwm297	7B
wms720	3A	cfd60	5B/6D	gwm333	7B
gwm2	3A	cfd10	5D	gwm644	7B
gwm5	3A	wmc233	5D	gwm302	7B
gwm480	3A	gwm192	5D	gwm274	7B
gwm666	3A	gwm358	5D	gwm344	7B
cfd79	3A,B,D	gwm583	5D	gwm577	7B
gwm533	3A,B,D	gwm174	5D	gwm611	7B
wms485	3B	gwm182	5D	gwm68	7B
gwm493	3B	gwm565	5D	gwm295	7D
gwm108	3B	wms82	6A	gwm44	7D
gwm547	3B	gwm459	6A	gwm111	7D
gwm247	3B	gwm494	6A	gwm37	7D
gwm340	3B	gwm169	6A	cfd175	7D

wmc326	3B,5B	gwm427	6A	cfd69	7D
cfd4	3B,D,4A	wmc553	6A		
wms456	3D	gwm132	6B		
cfd70	3D	gwm518	6B		

A DArT profiling of the parents and 134 doubled haploids yielded 398 polymorphic markers. A genetic map created using these markers showed poor marker saturation in the D genome in general (Fig 3.16). There are a few large gaps in this genetic map constructed using these markers and clustering of markers at some locations.

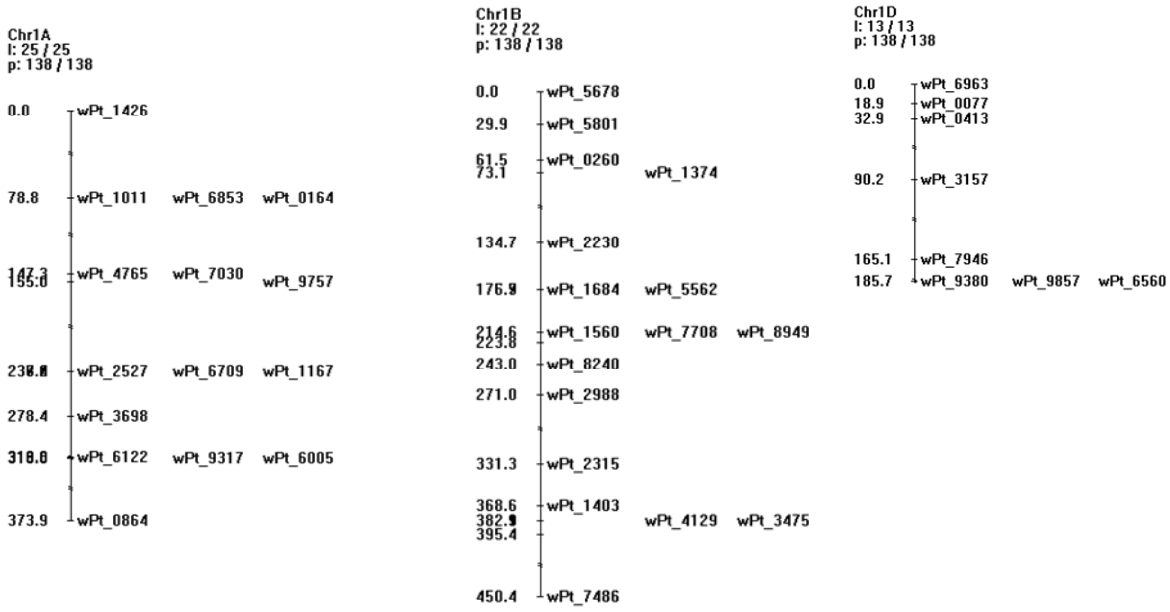
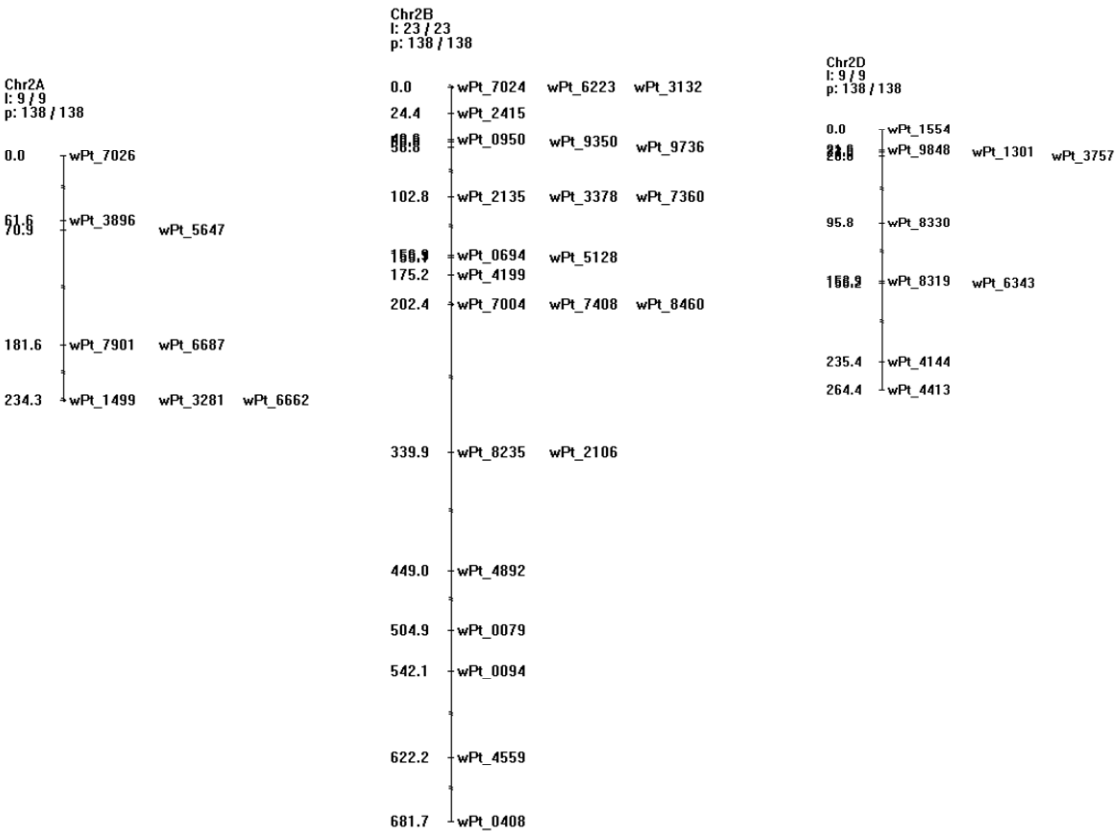


Fig 3.16 A statistical genetic map of wheat constructed using SSR markers and DArT markers derived from a doubled haploid population of F_1 hybrid (Sp5 x Otane). Doubled haploids were generated using wide hybridisation of the F_1 with maize.


Chr2A
l: 9 / 9
p: 138 / 138
0.0 wPt_7026
61.6 wPt_3896
70.9 wPt_5647
181.6 wPt_7901 wPt_6687
234.3 wPt_1499 wPt_3281 wPt_6662
Chr2B
l: 23 / 23
p: 138 / 138
0.0 wPt_7024 wPt_6223 wPt_3132
24.4 wPt_2415
wPt_0950 wPt_9350 wPt_9736
102.8 wPt_2135 wPt_3378 wPt_7360
wPt_0694 wPt_5128
175.2 wPt_4199
202.4 wPt_7004 wPt_7408 wPt_8460
339.9 wPt_8235 wPt_2106
449.0 wPt_4892
504.9 wPt_0079
542.1 wPt_0094
622.2 wPt_4559
681.7 wPt_0408
Chr2D
l: 9 / 9
p: 138 / 138
0.0 wPt_1554
wPt_9848 wPt_1301 wPt_3757
95.8 wPt_8330
wPt_8319 wPt_6343
235.4 wPt_4144
264.4 wPt_4413

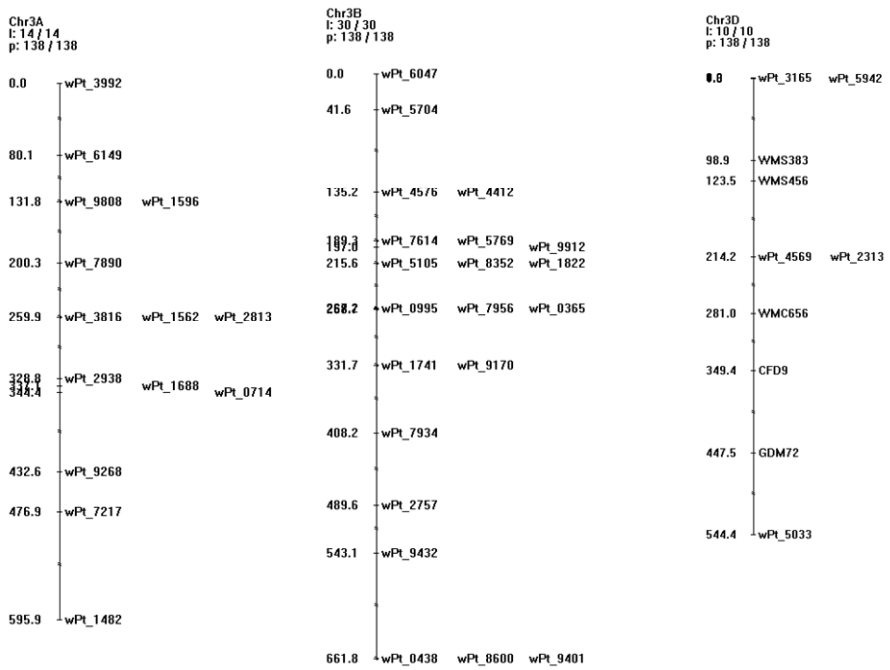


Fig 3.16 continued

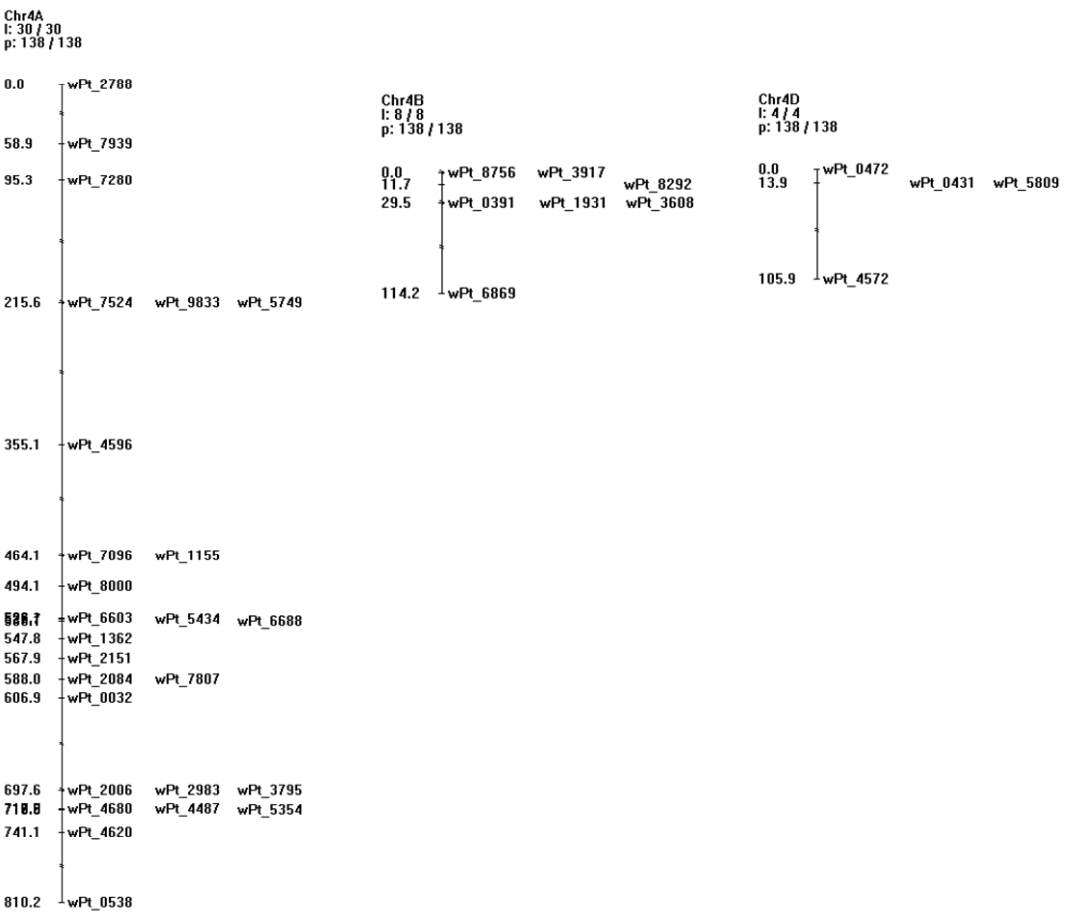


Fig 3.16 continued

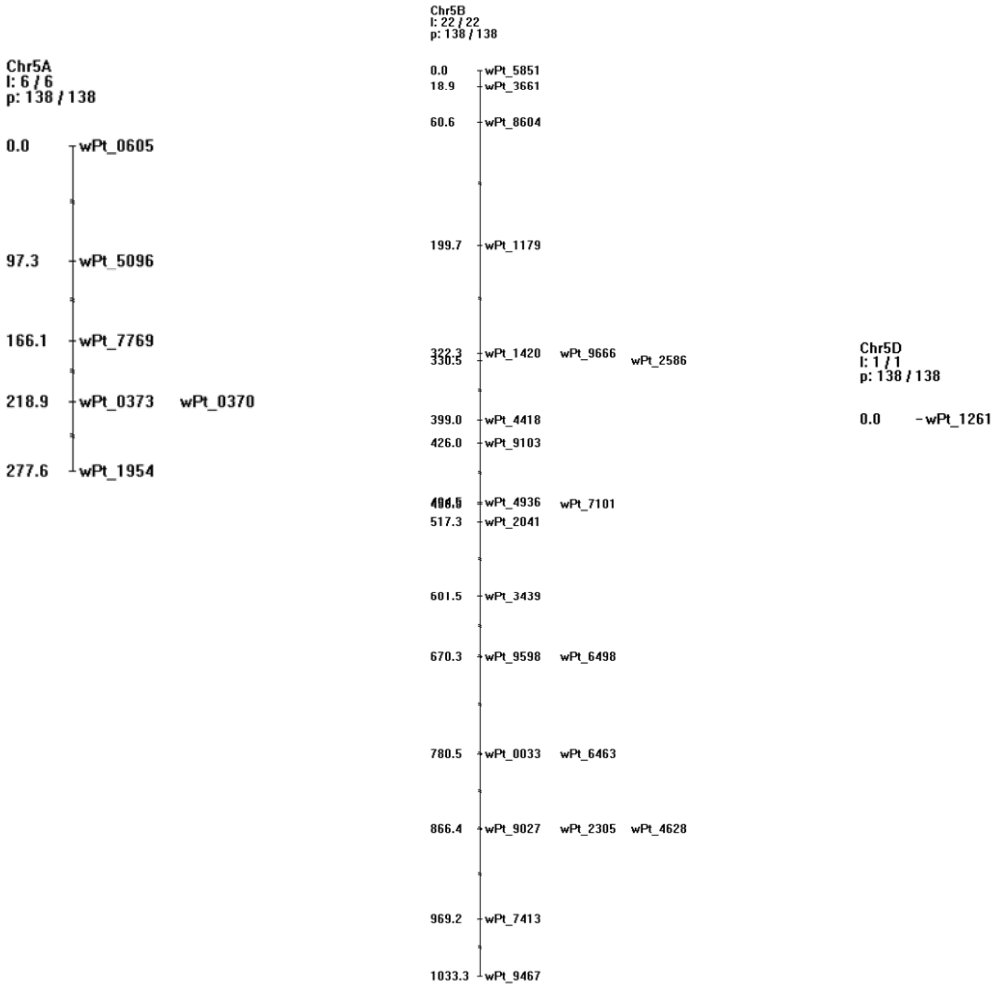


Fig 3.16 continued

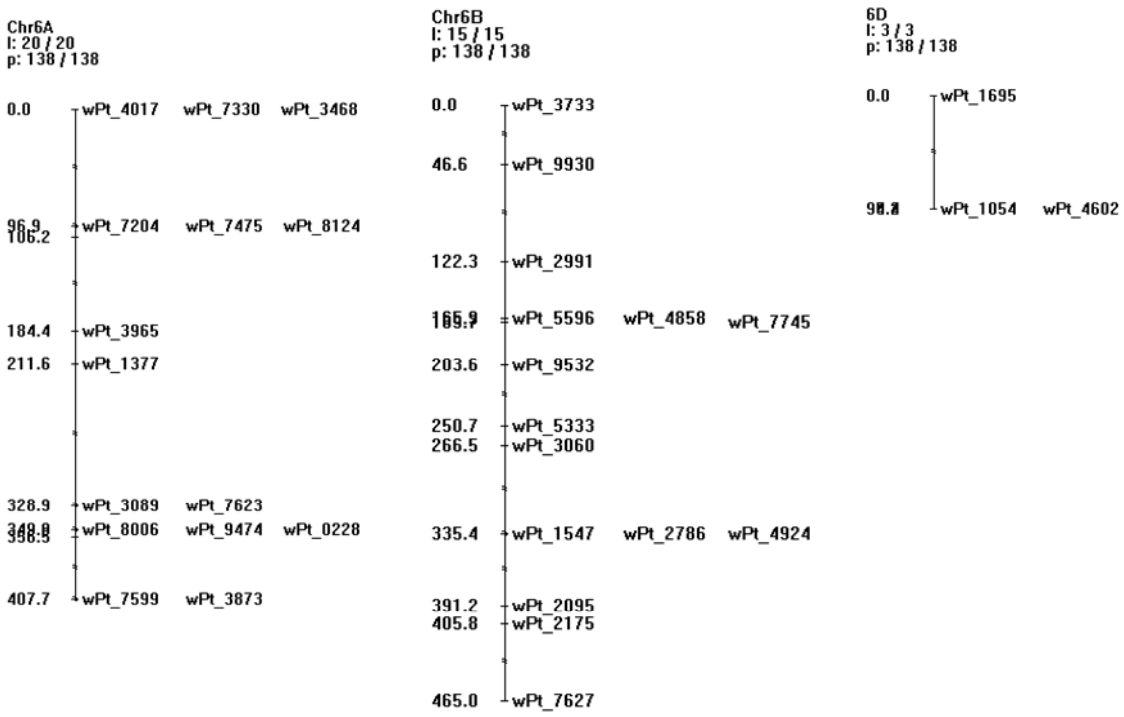


Fig 3.16 continued

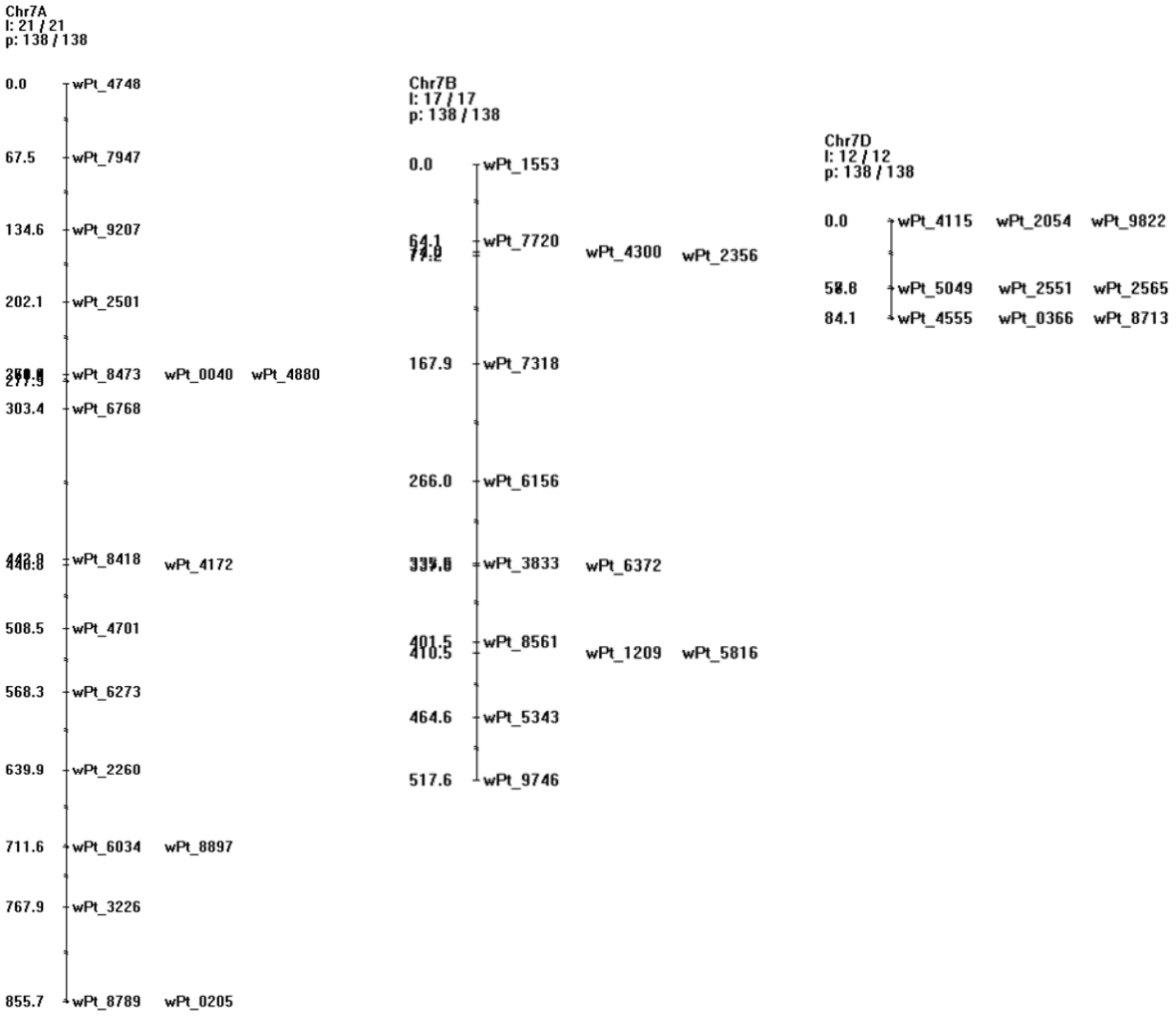


Fig 3.16 continued.

3.5. Regression analysis and quantitative trait loci (QTL) mapping

Results of the regression analysis (Appendix IV) performed using the MapManager QTX ® programme indicated that most of the variation associated with the complex sphaerococcum trait is contributed by loci around the SSR marker WMS 456 on chromosome 3D. The QTL analysis using MapManager QTX® yielded 12 QTLs (Fig 3.17) and the QTLs revealed co-segregation of all the traits in the sphaerococcum complex such as reduced plant height, head height, awn length, rounder grain shape, grain length, grain width, grain length /width ratio, grain depth, embryo length / width ratio, inner crease, outer crease and 1000 seed weight.

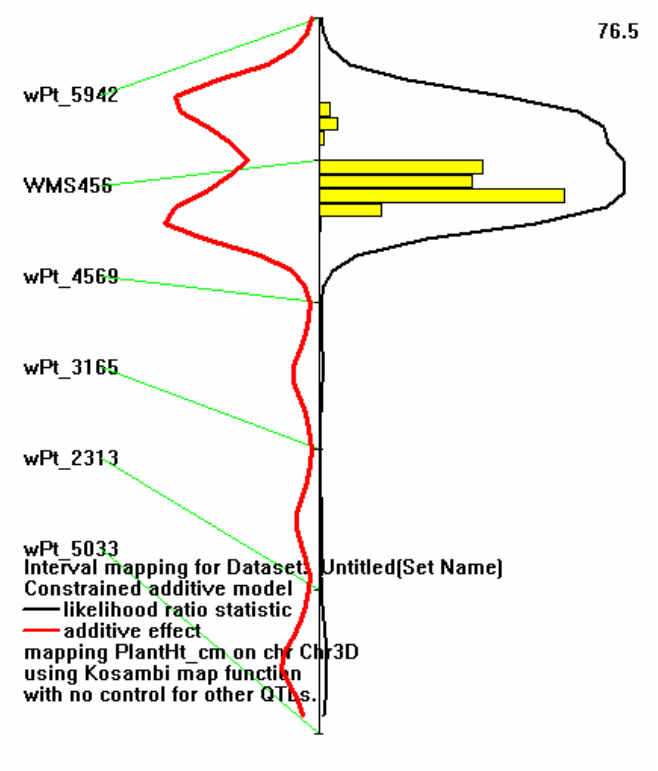


Fig 3.17a QTL for plant height. Regression analysis (P = 0.05) indicated that 42% of the variation associated with this trait is linked to chromosome 3D around the sphaerococcum specific locus WMS 456. The line in black (thinner line) indicates the likelihood ratio statistics (LRS) which is always positive to the right side. The line in colour (thicker) indicates the map position. This line could be on either side of the map depending on the sign of the regression coefficient.

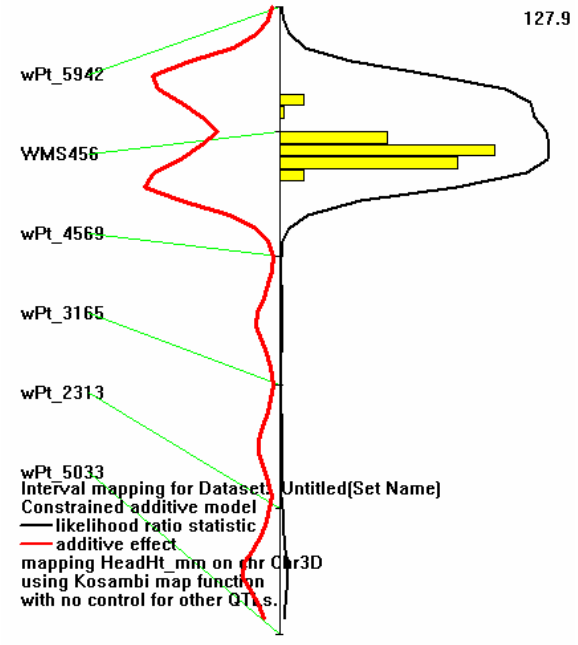


Fig 3.17b QTL for head height/ spike length. Regression analysis (P = 0.05) indicated that 60% of the variation associated with this trait is linked to the locus WMS 456 on chromosome 3D while +11% is linked to chromosome 5B.

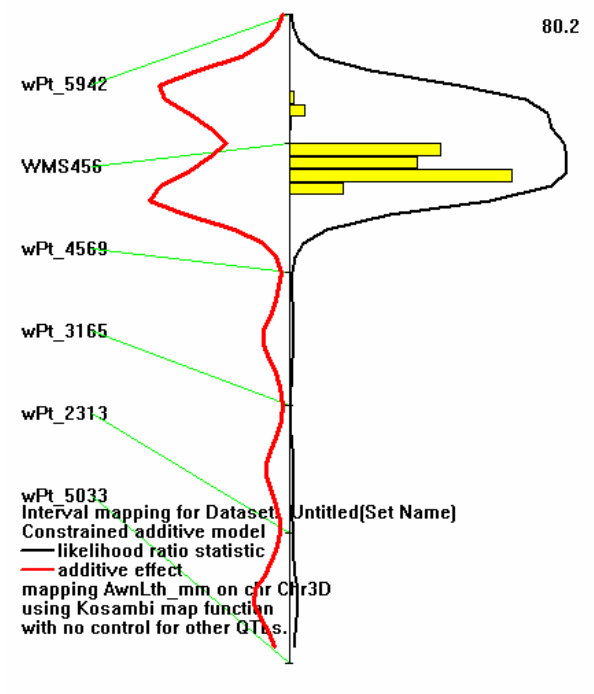


Fig 3.17c QTL for awn length. Regression analysis (P = 0.05) indicated that 42% of the variation associated with this trait is linked to the locus WMS 456 on chromosome 3D while 17% is linked to chromosome 2D and 13% to chromosome 4D.

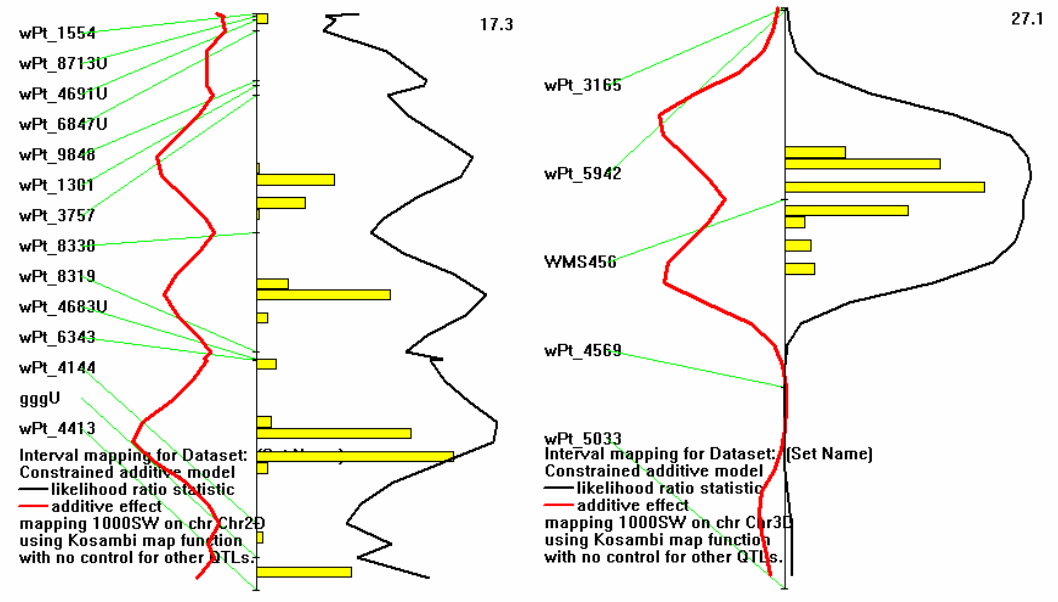


Fig 3.17d QTL for 1000 seed weight. Regression analysis (P = 0.001) indicated that 18% of the variation associated with this trait is linked to the locus WMS 456 on chromosome 3D while 52 is linked to loci on chromosome 2D.

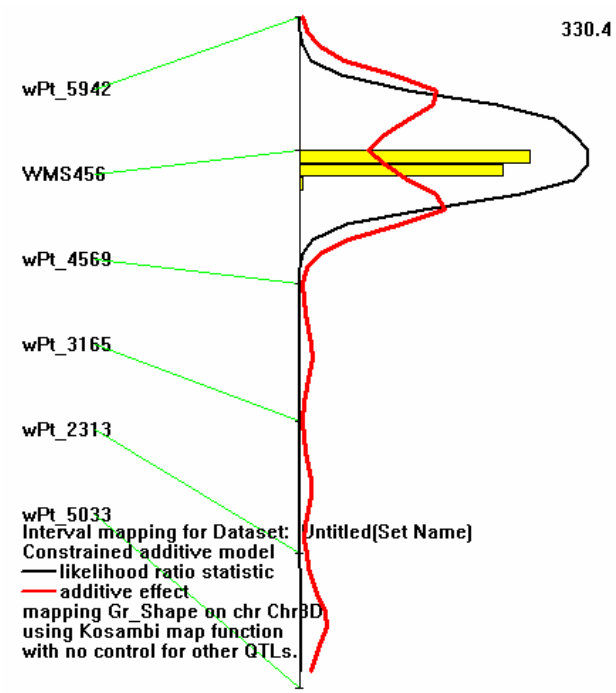


Fig 3.17e QTL for grain shape. Regression analysis (P = 0.05) indicated that most of the variation (91%) with this trait is linked to the locus WMS 456 on chromosome 3D.

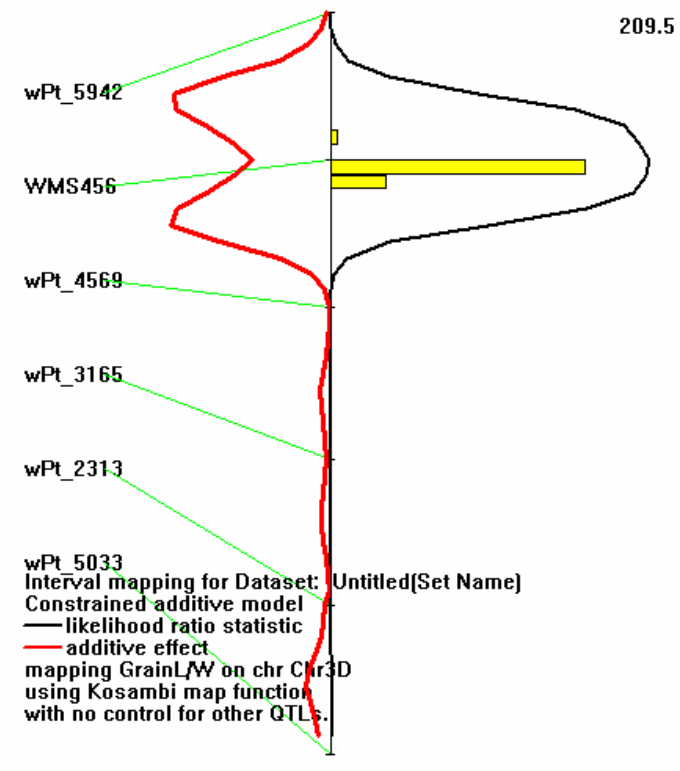


Fig 3.17f QTL for grain L/W. Regression analysis (P = 0.05) indicated that most of the variation (78%) associated with this trait is linked to the locus WMS 456 on chromosome 3D while 12 is linked to loci on chromosome 5B.

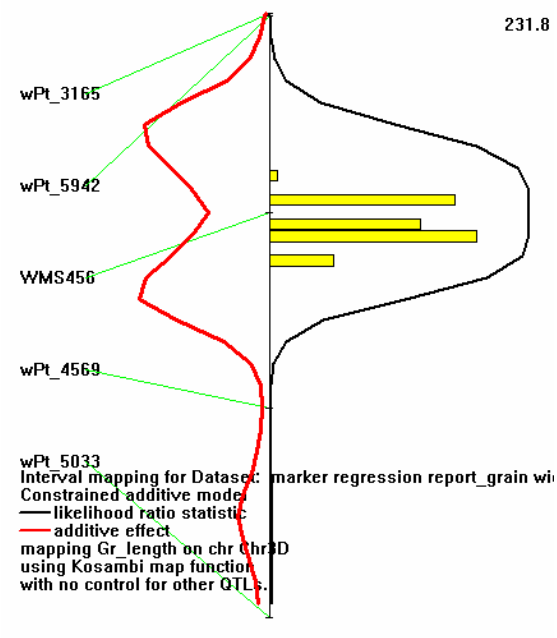


Fig 3.17g QTL for grain length. Regression analysis (P = 0.001) indicated that most of the variation (82%) associated with this trait is linked to the locus WMS 456 on chromosome 3D.

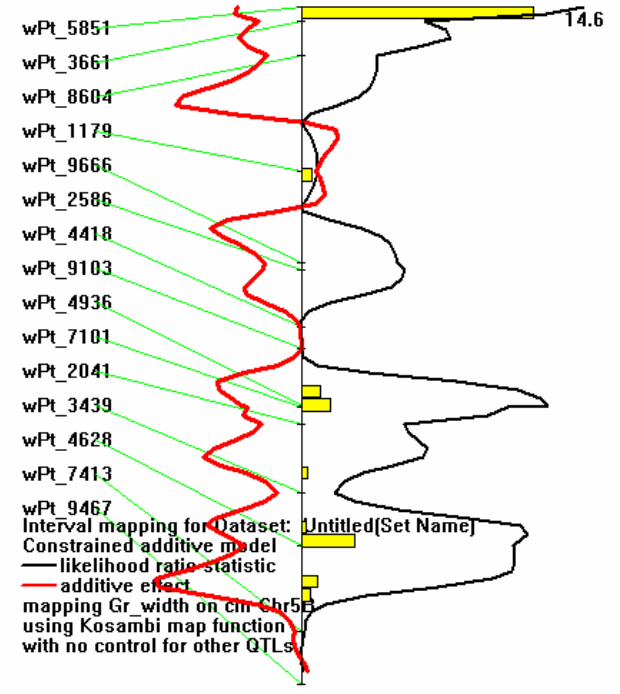


Fig 3.17h QTL for grain width. Regression analysis (P = 0.001) indicated that 34% of the variation associated with this trait is linked to loci on chromosome 5B while loci on chromosome 2D accounted for 12.5% of the variation.

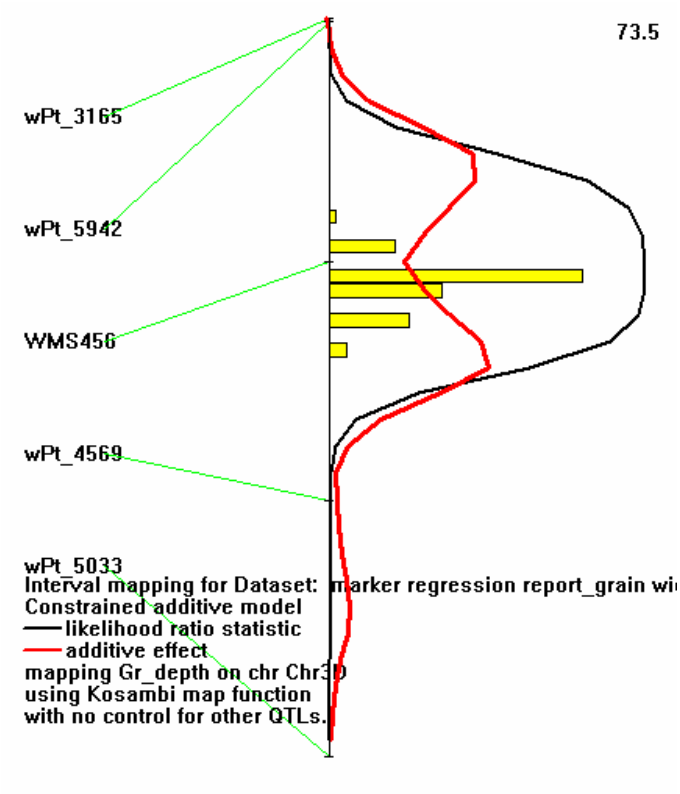


Fig 17i QTL for grain depth. Regression analysis (P = 0.001) for this trait showed that chromosome 3D accounted for 41% of all the variation associated with this trait.

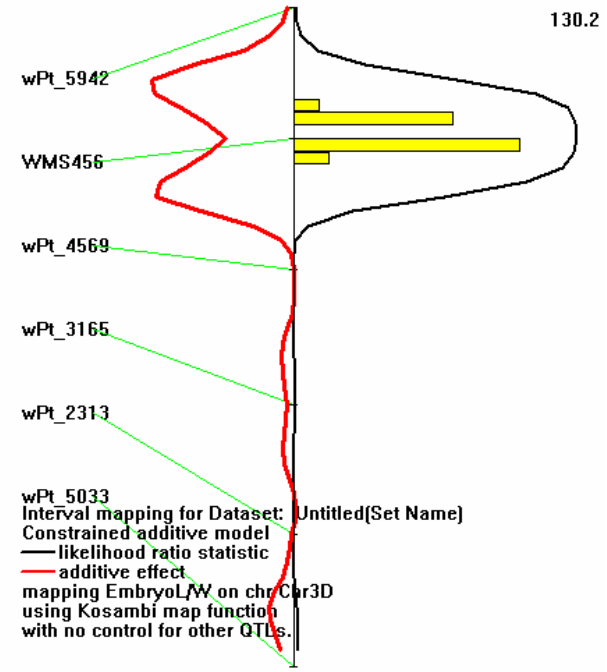


Fig 3.17j QTL for embryo L/W. Regression analysis (P = 0.05) indicated that loci on chromosome 3D accounted for much of the variation (61%) associated with this trait.

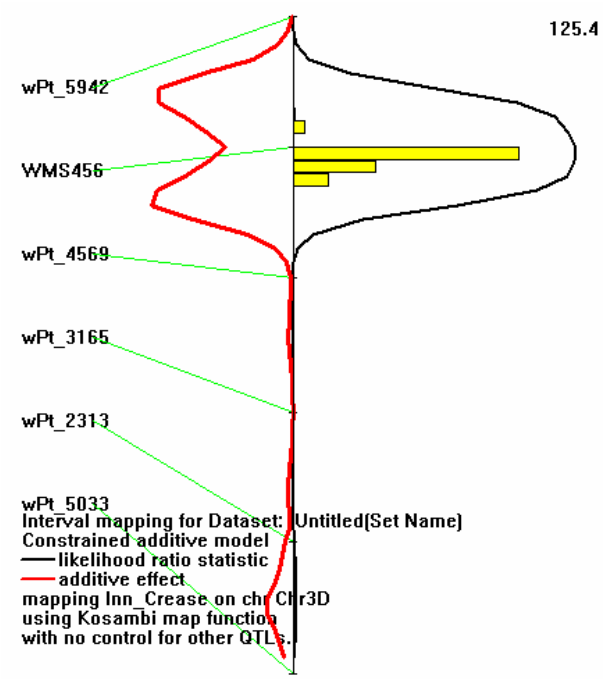


Fig 3.17k QTL for inner crease. Regression analysis (P = 0.05) indicated chromosome 3D accounted for 60% of the variation associated with this trait.

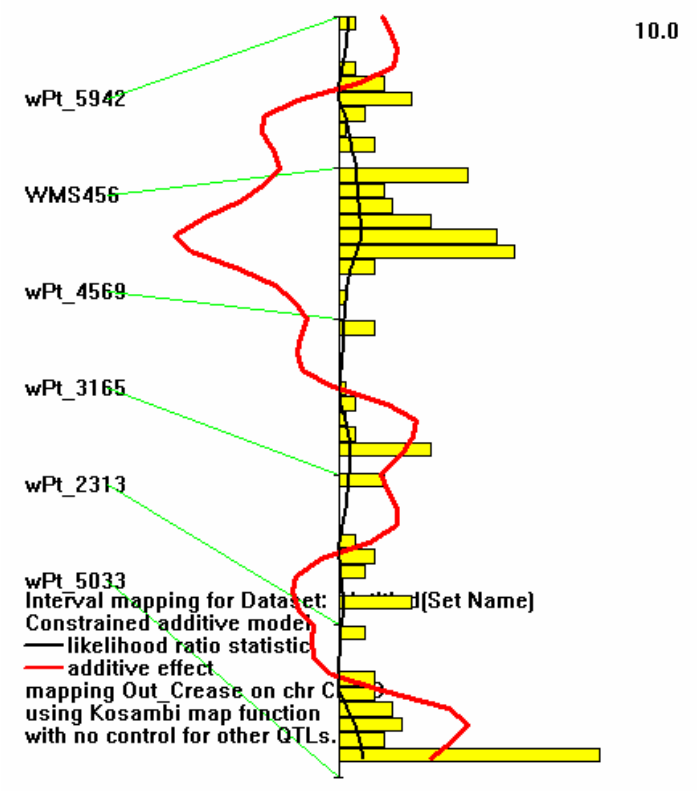


Fig 3.17l QTL for outer crease. From the regression analysis (P = 0.05) it appears that the outer crease is controlled by a number of loci on many chromosomes. Chromosome 7B accounted for most of the variation associated with this trait.

3.6. Mutation studies

T. sphaerococcum and *T. aestivum* mutated reciprocally when irradiated with gamma rays in a dose dependent fashion. Wheat seeds irradiated with gamma rays at 50 gray, 100 gray and 150 gray germinated and developed but irradiation at 200 gray was lethal. Otane and Sp5 seeds showed similar mutation frequency at comparable doses of gamma irradiation and produced chimeric plants with Otane-type and sphaerococcum-type tillers (Fig 3.18).

Fig 3.18 Chimeric wheat: whole plant (left) enlarged view of spikes (right) obtained through mutation with gamma rays (150 Grey). The initial seed was Sp5 so the "mutant" head is the one resembling T. aestivum. Chimeric plants with sphaerococcum-type heads were also obtained by mutating *T. aestivum* (Otane).

The aestivum-type heads obtained from chimeric plants generated by irradiating sphaerococcum-type seeds at 100 Grey and 150 Grey were grown for two generations in order to clean up any impure lines present in that lot. This seed lot is deposited with the Crop & Food Research, Lincoln, New Zealand.

CHAPTER FOUR

DISCUSSION

This research was initiated as a Ph D project but later converted to a Masters' project due to a change in the circumstances of the student. Therefore, the work presented here has several unanswered questions which can only be conclusively addressed with further research.

4.1. Seed germination

Seeds of Otane (*T. aestivum*) and Sp5 (*T. sphaerococcum*) exhibited similar rates of germination. Ambient temperature, moisture, oxygen and light are important external factors influencing seed germination (Bewley, 1997; Peng and Harberd, 2002; Koornneef et al. 2002). Field experiments by Khan et al. (1986) demonstrated the significance of soil temperature and moisture on the germination and emergence of wheat seeds. The quality of the seed, variety (genotype) of the wheat and ploidy of the wheat species are internal factors affecting wheat seed germination. Ahmad et al. (1998) reported variation in seed germination from 82% to 95% among the ten *T. aestivum* varieties they studied. Nyachiro et al. (2002) studied the effect of temperature on germination of ten spring wheat (*T. aestivum*) genotypes and found that there was a significant effect of genotype and temperature on the germination rate. Naylor (1993) studied the effect of parent plant nutrition on winter wheat cv. Avalon, and concluding that there was a positive correlation between parent plant nutrition (nitrogen) to seed size (seed quality) and subsequent germination. Akther et al. (1992) demonstrated that the storage of wheat seeds for long periods resulted in genetic abnormalities affecting cell division and subsequently germination rates. Seed storage conditions (grain moisture and ambient temperature) also influenced seed germination in wheat (Al-Yahya, 2001). In this study, we compared the germination rates of good quality seeds of hexaploid wheat (Otane and Sp5) which had been stored at similar conditions for one year. The seeds were incubated under favourable conditions of temperature and moisture to compare their germination rate. The germination of wheat seeds starts with imbibing water followed by synthesis of gibberellins in the embryo. Gibberellins in turn induce α-amylase production in the aleurone layer leading to the mobilisation of reserve food (starch) by the enzyme

action (Appleford and Lenton, 1997). Therefore, similar germination rates shown by Otane and Sp5 varieties in this experiment suggests similarities in their germination physiology.

4.2. Comparison of early seedling growth

Otane seedlings grew faster than Sp5 seedlings in the first week from germination (Table 3.1., Fig. 3.3 and 3.4). The mitotic index is a measure of the rate of cell division. In this study, Otane seedlings showed a greater mitotic index (18% more) in root tip cells compared to that of Sp5 seedlings. The cell cycle is regulated by the synergistic effect of cytokinins and auxins (John et al. 1993). Cytokinins control cell division in plants (Zhang et al. 1996; Mok and Mok, 2001; Yang et al. 2003). Auxins cause plant growth mainly by influencing cell elongation (Cleland, 1995). Moussavi-Nik et al. (1997) studied the remobilisation of nutrients in the wheat seed during germination and early growth, and concluded that there was a genotypic effect on the remobilisation of nutrients and subsequent growth of seedlings. Gibberellic acid also influences plant structure by promoting cell division and elongation (Sauter and Kende, 1992). In wheat, GA deficient and insensitive mutants (*Rht* mutants) demonstrate dwarfism due to an inability of cells to grow in length. GA_3 has little effect on cell division in dwarf (GA insensitive *Rht*) wheat and the dwarfism is caused by the inability of cells to grow in length (Keyes et al. 1989; Tonkinson et al. 1995). Miralles et al. (1998) compared the cellular dimensions of dwarf and double dwarf *Rht* wheat with a standard height variety (*rht* wheat) and concluded that the *Rht* genes lead to a reduction in cell length. However, the cell size was not compared in this study. Therefore, it is assumed that the faster early growth of Otane (*Rht 1*) seedlings compared to Sp5 seedlings observed in this study may be the result of a higher rate of cell division in Otane.

4.3 Further growth and development

Although Otane started anthesis one week earlier than Sp5, both varieties matured around the same time (Fig. 3.6 and 3.7). Development and maturation of wheat is controlled by the variety and the interaction of the genotype and environmental factors such as temperature, light intensity and photoperiod (Friend et al. 1962; Slafer and Rawson, 1994; Jamieson et al. 2007). Since both varieties were grown in the same glasshouse environment with similar nutrient conditions, the

observed difference in their development may point to genetic and physiological differences. Kato and Yamagata (1988) identified three genetic factors, namely photoperiod sensitivity (PS), vernalisation requirement (VR) and intrinsic earliness (IE) controlling earliness in wheat. Recent genetic analyses have discovered QTLs for the three genetic factors controlling the earliness trait (Shindo et al. 2003; Hanocq et al. 2004, 2007). Otane is a spring wheat having the *Rht1* gene. *Rht1*, *Rht2* and *Rht3* genes induce early flowering in wheat (Evans and Blundell, 1994). A spring wheat may not be affected by the VR factor. There are no reports on the vernalisation requirement of *T. sphaerococcum*. In this study, Otane and Sp5 were grown under the same photoperiod. Hence, the early flowering in Otane may not be due to a photoperiod factor. Therefore, it is possible that the intrinsic earliness factor may have caused early flowering in Otane compared to Sp5.

Wheat seeds undergo a period of growth of about 30-40 days depending on the variety, develop starchy tissue, accumulate starch and produce the aleurone layer, before the dehydration step leading to their maturation (Stoddard, 1999; Shewry and Halford, 2002). Wheat grain development is under genetic control and there is a large variation in development between varieties (Morell and Myers, 2005). The maturation process is also influenced by G x E interactions (Blum, 1998; Dupont and Altenbach, 2003; Triboi et al. 2003). Sphaerococcum seeds are smaller compared to the Otane seeds. This difference in their grain sizes may require less time for grain filling in Sp5 seeds leading to their rapid maturation.

4.4. Effect of plant hormones on early seedling growth

Otane and Sp5 showed similar shoot growth patterns in response to applied GA_3, IBA and BAP (Table 3.2; Fig.3.8 to 3.11). Hormones are most effective at optimum concentration. At supra-optimal concentrations they inhibit plant growth (Bradford and Trewavas, 1994) as observed in this study. Similar growth responses shown by Otane and Sp5 to the applied hormones may indicate their physiological similarity.

4.5. Effect of GA_3 on further growth of the seedlings

Application of GA_3 produced a transient elongation growth in Sp5 (Fig. 3.12). Various *Rht* genes influence the growth of wheat seedlings in different ways. Ellis et al. (2004) studied early seedling growth and elongation of first leaf in 20 wheat

cultivars with a known *Rht* background. They described variation in the coleoptile length and early growth (elongation of the first leaf) among the varieties studied. They found three different types of growth responses among these varieties. Wheat varieties with *Rht* 4, 5, 7, 8, 9, 12, and 13 showed no difference in the size of coleoptile and early growth in the presence and absence of GA. Varieties with *Rht* 1 and *Rht* 2 alleles showed a lack of response to applied GA_3. They also exhibited reduced leaf elongation rate and coleoptile length. Varieties with *Rht* 16 and 18 exhibited a reduction in coleoptile length and leaf growth but application of GA caused higher growth compared to other *Rht* types. In this study, varieties Chun-Mai 18 (*Rht 8*) and Rasp (*Rht* 13) were taller than variety V Me S3 (*Rht* 12) on day 15 (Fig. 3.13). Variety VMeS3 established a significant difference in growth by day 30 unlike varieties Chun-Mai 18 and Rasp which required about 35 days to establish a significant growth difference between the control and treatment. This difference in the response time may be linked to their bolting time. Varieties Otane (*Rht1*) and CS 971 (*Rht 2*) did not respond to the applied GA_3. Variety Icaro (*Rht 18*) showed largest difference in height between control and treatment which is similar to the observation of Ellis et al. (2004).

The growth response of variety Sp5 was a unique "seedling response" and its *Rht* status is not clear. The Sp5 seedlings responded to applied GA_3 for a short period (d 15 - 30) and maintained that difference in growth through to maturity. This is unlike varieties with *Rht1*, *Rht2*, *Rht8*, *Rht12*, *Rht 13* and *Rht18,* which were either completely unresponsive or continued to respond until maturity (Fig. 3.13). This seedling response is different from the observations of Ellis et al. (2004) who studied the growth of several *Rht* varieties. Knauber and Banowetz (1992) have described a unique growth response caused by phytochromes in *T. aestivum* cv. Tibet dwarf. Therefore, it appears that the *T. sphaerococcum* var. Sp5 may have a unique genetic mechanism that may be different from the *Rht* system and further studies are required to understand that mechanism.

4.6. Doubled haploid production

Wheat x maize hybridisation results in the formation of haploid embryos with one set of wheat chromosomes as a result of the elimination of maize chromosomes during the embryo development (Laurie and Bennett, 1989). There are many reports of using this method for doubled haploid wheat production (Campbell et al. 1998,

2000; Knox et al. 2000; Brazauskas et al. 2005; Ushiyama et al. 2007). Doubled haploid wheat were produced by wide hybridisation of F_1 (Otane x Sp5) with maize following Campbell et al. (1998). However, the frequency of the doubled haploids generated in the current study was lower compared to the report of Campbell et al. (1998). Campbell et al. (1998) had used controlled conditions of temperature and light intensity in a growth cabinet during the experiment. In this study the hybridisation experiment was carried out in a glasshouse where other wheat breeding experiments were in progress and severe infestation by leaf rust (*Puccinia triticina*) occurred. Temperature and light are critical factors influencing DH production (Campbell et al. 1998). Therefore, variable temperature and light present in the glasshouse during the course of this study may have affected the haploid embryo production and subsequent double haploid recovery. Severe rust infestation present in the glass house in which the crossing activity was carried out also caused a high rate of contamination when the embryos were cultured *in vitro.* More Otane-type plants compared to Sphaerococcum-type plants were present among the doubled haploid population (Appendix II). The reason for this distortion is not clear but it may be because of the lower rate of fertilisation of sphaerococcum-type ovaries by maize pollen or as a result of higher rate of abortion of sphaerococcum-type embryos during the development.

4.7. Response of the doubled haploid population to GA_3

The physiological basis of sphaerococcum wheat was further investigated using GA_3 as a probe. The double haploids varied considerably in their response to GA_3 (Fig 3.14). The plants demonstrating the greatest growth changes following the GA_3 application also showed sphaerococcum characters such as compact head, short awns and round grains. The *Rht* factor would also have been segregating but is unresponsive to the applied GA_3. Because doubled haploids are essentially homozygous for every locus, this segregation pattern suggested that the semidwarf phenotype and other characteristics of the complex sphaerococcum trait may be controlled by one gene or a tightly linked group of genes that inherit together as a unit.

4.8. Molecular marker analysis and gene mapping

Salina et al. (2001) considered microsatellite markers (SSR) as excellent molecular markers because (i) they are co-dominant, with different alleles on closely related lines; (ii) these markers can be analysed with relative ease using polymerase chain reaction (PCR); (iii) their analysis requires only small quantities of DNA from any plant tissue and (iv) they are present at high frequency ($1/10^4$ bp of the genome). Diversity arrays technology (DArT) is a cost effective microarray-hybridisation based technique that enables the simultaneous typing of several hundred polymorphic loci spread over the genome without the need for prior sequence information (Jaccoud et al. 2001; Wenzel et al. 2004). This technique was used recently to map the wheat genome (Akbari et al. 2006; Semagn et al. 2006).

A genome wide scan of the parents (Otane and Sp5) with 348 SSR markers showed that 49% of the markers are polymorphic between the parents (Table 3.4). However, only five of these polymorphic markers were located on chromosome 3D (WMS 383, WMS 456, WMC 656, CFD 9 and GDM 72). Due to lack of time only these markers were used to profile the mapping population of 150 doubled haploids in this study. These markers on chromosome 3D were prioritised for mapping work because the sphaerococcum gene (*s* gene), the gene of interest to this study, had been physically mapped to chromosome 3D by Rao (1977) and Koba and Tsunewaki (1978). Since the DArT profiling was carried out only on 134 DHs along with the parents (Otane and Sp5), SSR data for those 134 DHs along with the parents were used to construct the genetic map presented here.

This genetic map revealed that the D genome in particular has poor marker saturation. Similar reports of poor marker saturation on the D genome of wheat are on record (Roder et al. 1998; Song et al. 2005; Akbari et al. 2006). The clustering of DArT markers on some chromosomes was noticed. According to Semagn et al. (2006), clustering of DArT markers may be an indication of gene rich regions. There are many large gaps in this genetic map. These large gaps may be caused by the poor marker saturation or due to pseudo linkage. Pseudo linkage is an issue in statistical mapping when working with a data set that has poor marker saturation. Although it is possible to adjust the map distance using the mapmaking software by fixing the map distances, this approach was not attempted because an authentic map for a similar mapping population was not available in the literature for comparison. The Mapmanager® programme was used to make the genetic map but previous studies

(Roder et al. 1998; Somers et al. 2004; Akbari et al. 2006) have used Mapmaker® alone or in combination with Joinmap®. This difference in software may have also caused minor variations in the map.

The order of SSR markers in chromosome 3D of this map is in accordance with the high-density microsatellite consensus map of wheat (Somers et al. 2004). There are, however, differences in the map distances determined between the SSR markers. Similarly, there are some differences in the DArT marker orders and map distances in this map compared to the wheat DArT map of Akbari et al. (2006). Marker order and distance differences in the SSR marker orders on chromosome 3D also exist between the wheat SSR maps of Roder et al. (1998), Salina et al. (2001) and Somers et al. (2004). These differences in map distances and map order can be due to differences in the mapping populations used, marker saturation and the size of the data set (number of individuals in the mapping population) used for mapping. The mapping population of Roder et al. (1998) included only 70 recombinant inbred lines (RIL) from a wide cross between W-7984, an amphi-hexaploid wheat synthesised from *T. tauchii* (DD) and the *T. durum* (AABB) variety Altar with Mexican wheat variety Opata 85 and included 279 SSR markers. Somers et al. (2004) used 438 individuals from four different genetic backgrounds and 1,108 SSR markers to develop the high density consensus map. The current study used DHs (134 individuals) derived from the cross *T. aestivum* cv. Otane x *T. sphaerococcum* (Sp5) and only 5 SSR markers and 398 DArT markers.

4.9. QTL analysis

Molecular marker analysis including QTL analysis is of great significance to marker assisted selection and wheat breeding (Gupta et al. 1999; Kuchel et al. 2007). QTLs maps for several agronomic characters of bread are available in the literature (Campbell et al. 1999; Dholakia et al. 2003; Mori et al. 2005; Verma et al. 2005; Suenaga et al. 2005; Huang et al. 2006; Kumar et al. 2006; Narasimhamoorthy et al. 2006; Quarrie et al. 2006; Jordan et al. 2007; Kuchel et al. 2007a & b; Roder et al. 2008; Sun et al. 2008) and increasing numbers of QTLs are being mapped. We were interested in locating QTLs affecting the complex sphaerococcum phenotype including reduced height, compact head, short awns, 1000 seed weight, round grain shape and crease size. Most of these QTLs co-located close to the centromeric SSR marker WMS 456 (*gwm 456*) on chromosome 3D.

The marker regression report (Appendix IV) generated by the Mapmanager programme during the QTL analysis indicated that much of the variation associated with the complex sphaerococcum trait was associated with the SSR marker *gwm 456*. This agrees with the findings of Salina et al. (2000) who have mapped the sphaerococcoid genes *s1*, *s2* and *s3* close to the centromeric SSR marker *gwm 456* in a sphaerococcoid mutant population based on the physical mapping of the sphaerococcum gene (*sp* gene) to the centromeric region of chromosome 3D by Rao (1977), Koba and Tsunewaki (1978) and Singh (1987).

Plant height

This analysis indicated that 42% of the variation associated with plant height is linked to loci around the marker WMS 456 Other major contributors to this trait were located on chromosome 1A, 2D, 4B, 6A, 6D and 7D. Plant height in wheat is under polygenic control (Cadalen and Sourdille et al. 1998). The native sphaerococcum gene is mapped on chromosome 3D (Rao, 1977; Koba and Tsunewaki, 1978; Singh, 1987). Otane, the *T. aestivum* parent used in this study, carries *Rht* 1, a gene located on chromosome 4B (McVittie et al. 1978). Therefore, the effect of the sphaerococcum gene as well as the *Rht1* allele explains the apparent roles of regions on chromosome 4B and 3D in this analysis. The height reducing allele *Rht 8* is mapped on chromosome 2D of bread wheat (Korzun et al. 1998; Worland et al. 2001). Verma et al. (2005) identified three QTLs for plant height on chromosome 3D, 4B, and 4D respectively. Huang et al. (2006) identified one each significant QTLs for plant height on chromosome 4B, 4D, 5D and 7B. Spielmeyer et al. (2007) reported a significant QTL accounting for 11-16% of variation for plant height on chromosome 6A. Current results indicated a combined contribution of 14.7% to plant height by three loci on 6A. Nullisomy for most of the 21 chromosomes caused height reduction in wheat var. Chinese Spring although homoeologous groups 1, 2 and 4 were most effective in reducing the height (Sears 1954). The results of the current study also reflect those conclusions.

Spike length (Head height)

There are a few reports on QTLs for ear length on chromosome 1A, 2D, 4A (Li et al. 2002), 1A, 2D, 4A, 5A, 5B and 7D (Ma et al. 2007). These QTLs have a positive effect on the spike length and associated yield. In this study QTLs with minor

association with spike length were detected on chromosomes 2D, 3A, 4A, 5A, 5B, 6A and 7d. However, the most significant QTL with 60% of the variation associated with the trait was located on chromosome 3D around the sphaerococcum locus unlike previous studies. It is likely that this QTL located on 3D is responsible for the compactness of the sphaerococcum ear and the yield penalty associated with the sphaerococcum gene.

Awn length

The awned character in wheat is correlated with increased yield and grain weight under dry environmental conditions (Atkins and Norris, 1955; Weyrich et al. 1994; Cuthbert et al. 2008). The awning character in *T. aestivum* is controlled by the genes *Hd* located on chromosome 4A (Rao, 1981) and *B1* located on chromosome 5A and *B2* located on chromosome 6A respectively (Sears, 1954). Sourdille et al. (2002) studied the genes affecting awning in wheat using a doubled haploid population. They located two QTLs explaining 8.5 to 45.9% of the variation in this trait on chromosome 4B and Chromosome 6B. However, one QTL located on chromosome 3D explained much of the variation (44%) associated with the short awn character in this study. The marker regression analysis showed that several loci on chromosome 4A, 5B and 6A also explain small quantities of variations associated with this trait. A pleiotropic effect of the sphaerococcum gene may be indicated by the co-location of a major QTL for awn length with other QTLs for sphaerococcum component traits on chromosome 3D.

Grain shape

The sphaerococcum grain has a unique round shape. A single QTL located at sphaerococcum locus on chromosome 3D explained 91% of the variation associated with the round grain shape. Other QTLs associated with this trait were located on several chromosomes and had insignificant effects ranging from 3-6 %. Detection of a single QTL with a gross effect on this trait in this study indicates that this trait is under the control of the sphaerococcum gene. This argument is strengthened by the location of a single major QTL explaining 82% of the variation associated with the grain length as well.

Grain length

Campbell et al. (1999) reported QTLs for kernel length on chromosomes 3B, 2B, and 2D. Dholakia et al. (2003) reported QTLs for grain length with effects ranging from 3.3% 16.6% were reported on chromosome 7B, 5B, 6B, 2B and 2D. Five QTLs, on chromosome 1A, 1B, 2B, 4A and 4 B for kernel length were located by Sun et al. (2008). This study located only one QTL for the trait and the QTL was located close to the sphaerococcum locus on chromosome 3D. Since this QTL explained 82% of all the variation associated with the trait it can be we assume that this trait in sphaerococcum wheat is controlled by one gene.

Grain width

Campbell et al. (1999) located QTLs for kernel width on chromosomes 3D, 1A, 2A and 5A. Dholakia et al. (2003) mapped grain width QTLs to two loci on chromosome 2D. Sun et al. (2008) identified one each QTLs for kernel width on chromosome 2A, 5D and 6A. However, this study located QTLs for grain width on chromosome 5B with 26% of the variation and 2D with 9% of the variation associated with this trait. This trait may be under the control of multiple alleles because several QTLs associated with this trait were identified on different chromosomes.

Grain length/ width ratio

One QTL on Chromosome 3D explained 78% of the variation associated with this trait. Many loci on different chromosomes with a marginal contribution to the trait (3-4% of the variation) were also detected. Considering the significance of a single QTL along with the major QTLs associated with the round grain shape and grain length, it is assumed that the pleiotropic effects of the sphaerococcum gene is controlling the gross morphology of the spherical grain.

Grain depth

Most of the variation associated with this trait links to QTLs around the sphaerococcum locus on chromosome 3D (49% variation) and a minor QTL around the locus WPT 33 on chromosome 5B (9% variation). Therefore, it is likely that the pleiotropic effect of the sphaerococcum gene has a control on this trait as well.

Embryo length/ width ratio

Since one major QTL located around the sphaerococcum locus explains 61% of all the variation associated with this trait, it is assumed that this trait is also controlled by the pleiotropic effect of the sphaerococcum gene in the sphaerococcum wheat.

% Inner crease

A reduced crease may increase the yield of high quality flour because it can reduce the mixing of aleurone layer with white flour during milling. Sphaerococcum wheat is characterised by a small inner crease (Appendix II). One major QTL located around the sphaerococcum locus explaining 60% of the variation associated with this character indicates the possible pleiotropic effect of the sphaerococcum gene on this trait also.

1000 seed weight

1000 seed weight is a commonly used agronomic character influencing yield. Eighteen percent of the variation in this trait is linked to loci near the marker WMS 456 on 3D, although other loci around DArT marker wpt 7408 on chromosome 2B and DArT markers wpt 9848, wpt 1301, wpt 8319, wpt 6343, wpt 4413 on 2D had significant effects. Dholakia et al. (2003) reported grain weight QTLs on chromosome 2B and 2D with a combined effect of 13.2%. Quarrie et al. (2005) studied grain yield in wheat across a range of growing conditions and identified significant QTLs for thousand seed weight on chromosome 1B, 1D, 2A, 2B, 3A, 3B, 3D, 4A, 4B, 4D, 5A, 5B, 6B, 6D, 7B and 7D. Verma et al (2005) detected QTLs for seed weight on Chromsome 1B, 1D and 2B. Kumar et al. (2006) mapped QTLs for 1000 seed weight on chromosome 1A, 2B and 7A. A major QTL for grain weight on chromosome 7D was described by Huang et al. (2003), Narasimhamoorthy et al. (2006), Roder et al. (2008). *T. sphaerococcum* has low yield compared with *T. aestivum* (Percival, 1921, Ellerton, 1939; Singh, 1946). Recent breeding experiments at Crop & Food Research, Lincoln have confirmed the yield penalty caused by the sphaerococcum trait (Pers. Comm. Ross Bicknell and Steve Shorter) confirming the results of this study. Previous studies have shown that the complex sphaerococcum trait is inherited as one unit, possibly controlled by a single gene (*s*) and it was not possible to segregate apart the individual components of this complex trait (Percival, 1921; Ellerton, 1939; Singh, 1946). Rao (1977) concluded that the *s* gene may be located on the short or long arm of chromosome 3D close to the centromere. Koba and Tsunewaki (1978) mapped the *s* gene to the long arm of chromosome 3D at 5.2 cM from the centromere. Singh (1987) mapped the sphaerococcum gene (*s*) to the long arm of chromosome 3D using crosses of monosomic 3D of var. Pb. C591 (white seeded) and ditelocentric for 3D long arm (3D L) of var. Chinese Spring (red seeded) and red seeded variety of *T.*

sphaerococcum. According to this study the component traits in the sphaerococcum complex namely spherical grain and short spike characteristic are located at 5.4 cM from the centromere on the long arm of chromosome 3D. The locus for short peduncle is located at 10.58 cM from the centromere on the long arm of chromosome 3D. The QTL analysis results in this study showed that the component traits of the complex sphaerococcum trait (short culm, short awns, grain shape, short grain, shallow inner crease, low 1000 seed weight) co-locate around the sphaerococcum specific locus WMS 456 on chromosome 3D. Therefore, our QTL results are in accordance with the results of previous genetic studies.

4.10. Mutation studies

Gamma irradiation reciprocally mutated *T. aestivum* to *T. sphaerococcum* and *vice versa* in a dose dependent fashion producing chimeric plants. There are a few reports of generating sphaerococcoid wheat from *T. aestivum* using the chemical mutagens ethyl methane sulfonate (Swaminathan et al. 1963; Chopra, 2006) and hydroxylamine (Gupta and Swaminathan, 1967; Chopra, 2006). Sphaerococcoid mutants were also produced from *T. aestivum* following irradiation with UV rays, Gamma rays (Swaminathan et al. 1963) and neutrons (Bozzini, 1965). Therefore, this demonstration strengthens the suggestion of Ellerton (1939) that *T. sphaerococcum* originated from *T. aestivum* through a mutation.

However, there are no reports of obtaining *T. aestivum-type* plants from *T. sphaerococcum* through induced mutation. This agrees with the work of Sears (1947) using monosomics and nullisomics. Swaminathan et al (1963) postulated that the sphaerococcoid mutants produced by UV light and gamma rays were essentially similar to *T. sphaerococcum*. However, recent SSR mapping (Salina et al. 2000) of sphaerococcoid mutants generated by irradiating *T. aestivum* located three genes namely *s1*, *s2* and *s3* which were located on chromosomes 3A, 3B and 3D respectively unlike the sphaerococcum gene (*s*) which physically mapped to one locus on the long arm of chromosome 3D (Rao, 1977; Koba and Tsunewaki, 1978; Singh, 1987) in the native *T. sphaerococcum* population.

CONCLUSIONS

A comparison of *T. sphaerococcum* var. Sp5 seedlings with cv. Otane (*Rht*1) for their response to IBA, BAP and GA_3 showed similar patterns of response in the first week of growth. However, they showed a different response to applied GA_3 after 10 days of growth. The effect of GA_3 on growth of the Sp5 with *T. aestivum* varieties, Otane, (*Rht*1), CS 971 (*Rht* 2), Chun-Mai-18 (*Rht* 8), VMe S3 (*Rht* 12), Rasp (*Rht* 13) and Icaro (*Rht* 18) was carried out to understand the physiological basis of the short character in sphaerococcum wheat. The sphaerococcum wheat demonstrated a unique "*seedling response"* to applied GA_3 unlike other varieties studied. Therefore, it appears that the plant height in *T. sphaerococcum* may not be under the control of the *Rht* system but may involve another mechanism. Further studies are required to understand the physiological basis of the height factor in *T. sphaerococcum*. Therefore, the hypothesis that *T. sphaerococcum* and dwarf varieties of *T. aestivum* are physiologically similar may not be true.

A doubled haploid population (150 individuals) developed through wide hybridisation of F_1 (Ot x Sp5) with maize was used for genetic analysis. A genome-wide scan of Otane and Sp5 varieties using SSR markers showed only 49% polymorphism (169 markers) between these parental varieties. However, a DArT profiling of the DH population yielded 398 polymorphic markers. A genetic map created using these molecular markers exhibited large gaps in the map. Clustering of DArT markers at particular regions on some chromosomes was noted. A QTL analysis detected one each major QTLs for most of the component traits in the complex sphaerococcum trait around sphaerococcum locus on chromosome 3D. This result suggested a possible pleiotropic effect of the sphaerococcum gene on all the component traits of the sphaerococcum character. The QTL results indicated that it may not be possible to separate the favourable characters of *T. sphaerococcum* from its unfavourable characters through mutation because the complex sphaerococcum trait may be under a pleiotropic control of the putative sphaerococcum gene.

Induced mutation using gamma rays (100, 150 and 200 gray) resulted in reciprocal mutations between sphaerococcum-type and aestivum-type wheat. This observation supported the hypothesis that *T. sphaerococcum* originated from a mutation in *T. aestivum.*

CITED REFERENCES

Achard P, Vriezen WH, Van Der Straeten D, Harberd NP (2003) Ethylene regulates Arabidopsis development via the modulation of DELLA protein growth repressor function. Plant Cell 15: 2816-2825

Acquaah G (2007) Principles of Plant Genetics and Breeding. Blackwell Publishing Ltd, Malden, USA.

Ahmad S, Anwar M, Ullah H (1998) Wheat seed presoaking for improved germination. Journal of Agronomy and Crop Science 181: 125-127

Akbari M, Wenzl P, Caig V. Carling J, Xia L, Yang S, Uszynski G, Mohler V, Lehmensiek A, Kuchel H, Hayden M J, Howes N, Sharp P, Vaughan P, Rathmell B, Huttner E, Kilian A (2006) Diversity arrays technology (DArT) for high-throughput profiling of the hexaploid wheat genome. Theoretical and Applied Genetics 113: 1409-1420

Akhter FN, Kabir G, Mannan MA, Shaheen NN (1992) Aging effect of wheat and barley seeds upon germination, mitotic index and chromosomal damage. Journal of Islamic Academy of Sciences 5 (1): 44-48

Allaby RG, Brown TA (2003) AFLP data and the origins of domesticated crops. Genome 46: 448-453

Allan RE (1989) Agronomic comparisons between *Rht_1* and *RHt_2* semidwarf genes in winter wheat. Crop Science 29: 1103-1108

Almouslem AB, Jauhar PP, Peterson TS, Bommineni VR, Rao MB (1998) Haploid durum wheat production via hybridization with maize. Crop Science 38: 1080-1087

Alvey L, Harberd NP (2005) DELLA proteins: integrators of multiple plant growth regulatory inputs? Physiologia Plantarum 123: 153-160

Al-Yahya SA (2001) Effect of storage conditions on germination in wheat. Journal of Agronomy and Crop Science 186: 273-279

Appleford NEJ, Lenton JR (1997) Hormonal regulation of α- amylase gene expression in germinating wheat (*Triticum aestivum)* grains. Physiologia Plantarum 100: 534-542

Atkins IM, Norris MJ (1955) The influence of awns on yield and certain morphological characters of wheat. Agronomy Journal 47: 218-220

Barclay IR (1975) High frequencies of haploid production in wheat (*Triticum aestivum*) by chromosome elimination. Nature 256: 410- 411

Bar-Yosef O (1998) The natufian culture in the Levant, threshold of the origin of agriculture. Evolutionary Anthropology 6: 159-177

Bell GDH (1987) The history of wheat cultivation. *In*: FGH Lupton, ed, Wheat Breeding: Its Scientific Basis. Chapman and Hall Ltd, London, p 31-47

Bewley JD (1997) Seed germination and dormancy. The Plant Cell 9: 1055-1066

Berlyn GP, Miksche JP (1976) Botanical Microtechnique and Cytochemistry. The Iowa State University Press, Ames, Iowa, p 306-307

Bicknell R, Josekutty PC (2006) Defining genetics and physiology of *Triticum sphaerococcum. In* CF Mercer, ed, Breeding for Success: Diversity in Action. Proceedings of the 13th Australasian Plant Breeding Conference, Christchurch, New Zealand 18-21 April 2006. p 937-945

Bjormstand AA, Skinnes H, Thorsen K (1993b) Comparisons between doubled haploid lines produced by anther culture, the Hordeum bulbosum-method and lines produced by single seed descent in barley crosses. Euphytica 66:135- 144

Blake NK, Lehfeldt BR, Lavin M, Talbert LE (1999) Phylogenetic reconstruction based on low copy DNA sequence analysis in an allpolyploid: the B genome of wheat. Genome 42: 351-360

Blum A (1998) Improving wheat grain filling under stress by stem reserve mobilisation. Euphytica 100: 77-83

Bonnett DG, Rebetzke GJ, Spielmeyer W (2005) Strategies for efficient implementation of molecular markers in wheat breeding. Molecular Breeding 15: 75-85

Borner A, Plaschke J, Korzun V, Worland AJ (1996) The relationships between dwarfing genes of wheat and rye. Euphytica 89: 69-75

Borner A, Schumann E, Furste A, Coster H, Leithold B, Roder MS, Weber WE (2002) Mapping of quantitative trait loci determining agronomic important characters in hexaploid wheat (*Triticum aestivum* L.). Theoretical and Applied Genetics 105: 921-936

Borojevic, S (1990) Principles and methods of plant breeding. Developments in Crop Science Vol.17, Elsevier Science, Amsterdam

Bozzini A (1965) Sphaerococcoid, a radiation induced mutation in *Triticum durum* Desf. Use of Induced Mutations in Plant Breeding. Pergamon Press, London, p 375-383

Bradford KJ, Trewavas AJ (1994) Sensitivity thresholds and variable time scales in plant hormone action. Plant Physiology 105: 1029-1036

Brammer SP, Fernandes MIB, Barcellos AL, Milach SCK (2004) Genetic analysis of adult-plant resistance to leaf rust in a double haploid wheat (*Triticum aestivum* L. em Thell) population. Genetics and Molecular Biology 27: 432- 436

Brazauskas G, Paoakinskiene I, Jahoor A (2004) AFLP analysis indicates no introgression of maize DNA in wheat x maize crosses. Plant Breeding 123: 117- 121

Brazauskas G, Paoakinskiene I, Ruzgas V (2005) Improved approaches in wheat x maize crossing for wheat doubled haploid production. Biologia Plantarum 4:15-18

Busino L, Donzelli M, Chiesa M, Guardavaccaro D, Ganoth D, Dorello NV, Hershko A, Pagano M, Draetta GF (2003) Degradation of Cdc25A by α- TrCP during S phase and in response to DNA damage. Nature 426: 87- 91

Cadalen T, Sourdille P, Charmet G, Tixier MH, Gay G, Boeuf C, Bernard S, Leroy P, Bernard M (1998) Molecular markers linked to genes affecting plant height in wheat using a doubled- haploid population. Theoretical and Applied Genetics 96: 933- 940

Caldwell KS, Dvorak J, Lagudah ES, Akhunov EN, Luo M, Wolters P, Powell W (2004) Sequence polymorphism in polyploid wheat and their D-genome diploid ancestor. Genetics 167: 941-947

Campbell KG, Bergman CJ, Gualberto DG, Anderson JA, Giroux MJ, Hareland G, Fulcher RG, Sorrells ME, Finney PL (1999) Crop Science 39: 1184-1195

Campbell AW, Griffin WB, Conner AJ, Rowarth JS, Burritt DJ (1998) The effects of temperature and light intensity on embryo numbers in wheat doubled haploid production through wheat x maize crosses. Annals of Botany 82: 29-33

Campbell AW, Griffin WB, Burritt DJ, Conner AJ (2000) Production of wheat doubled haploids via wide crosses in New Zealand wheat. New Zealand Journal of Crop and Horticultural Science 28: 185-194

Chantret N, Salse J, Sabot F, Rahman S, Bellec A, Laubin B, Dubois I, Dossat C, Joudrier P, Gautier M, Cattolico L, Becket M, Aubourg S, Weissenbach J, Caboche M, Bernard M, Leroy P, Chalhoub B (2005) Molecular basis of evolutionary events that shaped the hardness locus in diploid and polyploid wheat species (*Triticum* and *Aegilops*) The Plant Cell 17: 1033- 1045

Chaudhary HK, Sethi GS, Singh S, Pratap A, Sharma S (2005) Efficient haploid induction in wheat by using pollen of *Imperata cylindrica*. Plant Breeding 124 (6): i – iv.

Cheng H, Qin L, Lee S, Fu X, Richards DE, Cao D, Luo D, Harberd NP, Peng J (2004). Gibberellin regulates Arabidopsis floral development via suppression of DELLA protein function. Development 131: 1055-1064

Chopra VL, Swaminathan MS (1966) Mutagenic efficiency of individual and combined treatments of ethyl-methane-sulfonate and hydroxylamine in emmer wheat. Indian Journal of Genetics and Plant Breeding 26: 59-62

Chopra VL (2005) Mutagenesis: Investigating the process and processing the outcome for crop improvement. Current Science 89: 353-359

Ciaffi M, Dominici L, Umana E, Tanzarella OA, Porceddu E (2000) Restriction fragment length polymorphism (RFLP) for protein disulfide isomerase (PDI) genes sequences in *Triticum* and *Aegilops* species. Theoretical and Applied Genetics 101: 220- 226

Cleland RE (1995) Auxin and cell elongation. *In* PJ Davies, ed, Plant Hormones and Their Role in Plant Growth and Development, Ed 2. Kluwer, Boston, pp 214-227

Collard BCY, Grams RA, Bovill WD, Percy CD, Jolley R, Lehmensiek A, Wildermuth G, Sutherland MW (2005) Development of molecular markers for crown rot resistance in wheat: mapping of QTLs for seedling resistance in a '2-49 x 'Janz' population. Plant Breeding 124: 532- 537

Cross RJ, Wallace AR (1994) Loss of genetic diversity from heterogeneous self-pollinating genebank accessions. Theoretical and Applied Genetics 88: 885-890

Cuthbert JL, Somers DJ, Brule-Babel AL, Brown PD, Crow GH (2008) Molecular mapping of quantitative trait loci for yield and yield components in spring wheat (*Triticum aestivum* L.). Theoretical and Applied Genetics 117: 595-608

David JL, Dusautoir JC, Raynauld C, Roumet P (1999) Heritable variation in the ability to produce haploid embryos via pollination with maize and embryo rescue in durum wheat. Genome 42: 338-342

Davies, PJ (1995) The plant hormones: Their nature, occurrence, and functions. *In* PJ Davies, ed, Plant Hormones: Physiology, Biochemistry and Molecular Biology, Kluwer, Boston, p 3-38

Dill A, Jung HS, Sun TP (2001) The DELLA motif is essential for gibberellin–induced degradation of RGA. Proceedings of the National Academy of Sciences, USA, 98: 14162-14167

Dill A, Sun TP (2001) Synergistic derepression of gibberellin signaling by removing RGA and GAI function in *Arabidopsis thaliana*. Genetics 159: 777- 785

Dill A, Thomas SG, Hu J, Steber CM, Sun TP (2004) The Arabidopsis F-box protein SLEEPY 1 targets gibberellin signaling repressors for gibberellin-induced degradation. Plant Cell 16: 1392- 1405

Dholakia BB, Ammiraju JSS, Singh H, Lagu MD, Roder MS, Rao VS, Dhaliwal HS, Ranjekar PK, Gupta VS (2003) Molecular marker analysis of kernel size and shape in bread wheat. Plant Breeding 122: 392-395

Dunwell JM (1985) Anther and ovary culture. *In* SWJ Bright, MGK Jones, eds, Cereal Tissue and Cell Culture, Martinus Nijhoff/ Dr. W. Junk Publishers, the Netherlands, p 1-44

Dupont FM, Altenbach SB (2003) Molecular and biochemical impacts of environmental factors on wheat grain development and protein synthesis. Journal of Cereal Science 38: 133-146

Dvorak J, McGuire PE, Cassidy B (1988) Apparent sources of the A genomes of wheats inferred from polymorphisms in abundance and restriction fragment length of repeated nucleotide sequences. Genome 30: 680- 689

Dvorak J, Diterlizzi P, Zhang H-B, Resta P (1993) The evolution of polyploid wheats: identification of the A genome donor species. Genome 36: 21-31

Dvorak, J., Luo, M. C., Yang, Z. L., and Zhang, H. B. (1998). The structure of the *Aegilops tauschii* genepool and the evolution of hexaploid wheat. Theoretical and Applied Genetics 97: 657- 670

Evans LT, Blundell C (1994) Some aspects of photoperiodism in wheat and its wild relatives. Australian Journal of Plant Physiology 21: 551-562

Ellerton S (1939) The origin and geographical distribution of *Triticum sphaerococcum* prec. and its cytogenetical behaviour in crosses with *T. vulgare* Vill. Journal of Genetics 38: 307-324

Ellis MH, Rebetzke GJ, Chandler P, Bonnett D, Spielmeyer W, Richards RA (2004) The effect of different height reducing genes on the early growth of wheat. Functional Plant Biology 31: 583-589

Fan L, Feng X, Wang Y, Deng XW (2007) Gibberellin signal transduction in rice. Journal of Integrative Biology 49(6): 731-741

Feldman M (2001) Origin of cultivated wheat. *In* AP Bonjean, WJL Angus, eds, The World Wheat Book: A History of Wheat Breeding, Lavoisier Publishing, London, p 3-56

Feldman M, Levy AA (2005) Allopolyploidy- a shaping force in the evolution of wheat genomes. Cytogenetic and Genome Research 109: 250-258

Flintham JEF, Borner A, Worland AJ, Gale MD (1997) Optimising wheat grain yield: effects of *Rht* (gibberellin- insensitive) dwarfing genes. Journal of Agricultural Science 128: 11-25

Fox RL, Hayden MJ, Mekuria G, Eglinton JK (2005) Cost analysis of molecular techniques for marker assisted selection within a barley breeding programme. *In:* Proceedings of the 12th Australian Annual Barley Technical Symposium, Hobart, Australia

Friend DJC, Helson VA, Fisher JE (1962) Leaf growth in Marquis wheat as regulated by temperature, light intensity, and day length. Canadian Journal of Botany 40: 1299-1311

Fu X, Harberd NP (2003) Auxin promotes Arabidopsis root growth by modulating gibberellin response. Nature 421: 740-743

Fu X, Richards DE, Ait-Ali T, Hynes LW, Ougham H, Peng J, Harberd NP (2002) Gibberellin-mediated proteasome-dependent degradation of the barley DELLA protein SLN1 repressor. The Plant Cell 14: 3191-3200

Fu X, Richards DE, Fleck B, Xie D, Burton N, Harberd NP (2004) The Arabidopsis mutant sleepy1$^{gar2\text{-}1}$ protein promotes plant growth by increasing the affinity of the SCF^{SLY1} E3 ubiquitin ligase for DELLA protein substrates. The Plant Cell 16: 1406-1418

Gale MD, Youssefian S (1985) Dwarfing genes in wheat. *In W Russel, ed,* Progress in Plant Breeding, Butterwoths, London, p 1-35

Galili S, Avivi Y, Millet E, Feldman M (2000) RFLP based analysis of three RbcS subfamilies in diploid and polyploid species of wheat. Molecular and General Genetics 263: 674- 680

Garcia-Ilamas C, Martin A, Ballesteros J (2004) Differences among auxin treatments on haploid production in durum wheat x maize crosses. Plant Cell Reports 23: 46-49

Giles RJ, Brown TA (2006) *Glu-Dye* allele variations in *Aegilops tauschii* and *Triticum aestivum*: implications for the origins of hexaploid wheats. Theoretical and Applied Genetics 112: 1563- 1572

Gill BS, Kimber G (1974) Giemsa C- Banding and the evolution of wheat. Proceedings of the National Academy of Sciences, USA 71(10): 4086- 4090

Gill BS, Appels R, Botha-Oberholster AM, Buell CB, Bennetzen JL, Chalhoub B, Chumley F, Dvorak J, Iwanaga M, Keller B, Li W, McCombie WR, Ogihara Y, Quetier F, Sasaki T (2004) A workshop report on wheat genome sequencing: International genome research on wheat consortium. Genetics 168: 1087-1096

Gilpin MJ (1996) Characterisation of plants regenerated from barley (*Hordeum vulgare* L.)–H bulbosum L. hybrids. PhD Thesis. Lincoln University, Lincoln, p 31-37

Gomi K, Sasaki A, Itoh H, Ueguchi-Tanaka M, Ashikari M, Kitano H, Matsuoka M (2004) GID2, an F-box subunit of the SCF E3 complex, specifically interacts with phosphorylated SLR1 protein and regulates the gibberellin-dependent degradation of SLR1 in rice. Plant Journal 37: 626- 634

Golovnina KA, Glushkov SA, Blinov AG, Mayorov VI, Adkison LR, Goncharov NP (2007). Molecular phylogeny of the genus *Triticum* L. Plant Systematics and Evolution 264: 195-216

Griffin WB (1987) Outcrossing in New Zealand wheats measured by occurrence of purple grain. New Zealand Journal of Agricultural Research 30: 287-290

Gu YQ, Coleman-Derr D, Kong X, Anderson OD (2004) Rapid genome evolution revealed by comparative sequence analysis of orthologous regions from four Triticeae genomes. Plant Physiology 135: 1-12

Gubler F, Chandler PM, White RG, Llewellyn DJ, and Jacobsen JV (2002) Gibberellin signaling in barley aleurone cells. Control of SNL1 and GAMYB expression. Plant Physiology 129: 191-200

Gupta N, Swaminathan MS (1967) An induced sphaerococcoid mutant in *Triticum dicoccum*. Current Science, 36: 19

Gupta PK, Varshney RK, Sharma PC, Ramesh B (1999) Molecular markers and their applications in wheat breeding. Plant Breeding 118: 369-390

Guzy-Wrobelska J, Labocha-Pawlowska A, Kwasniewski M, Szarejko I (2007) Different recombination frequencies in wheat doubled haploid populations obtained through maize pollination and anther culture. Euphytica 156: 173-183

Hanocq E, Niarquin M, Heumez E, Rousset M, Le Gouis J (2004) Detection and mapping of QTL for earliness components in a bread wheat recombinant inbred lines population. Theoretical and Applied Genetics 110: 106-115

Hanocq E, Laperche A, Jaminon O, Laine AL, Le Gouis J (2007) Most significant genome regions involved in the control of earliness traits in bread wheat, as revealed by QTL meta-analysis. Theoretical and Applied Genetics 114: 569-584

Harlan JR (1981) The Early History of Wheat. *In* LT Evans, WJ Peacoke, eds, Wheat Science Today and Tomorrow. Cambridge University Press, Cambridge, p 1-9

Harris DR (2005) Origins and spread of agriculture *In* G Prance, M Nesbitt, eds, Routledge, New York, p 13-26

Hayden MJ, Nguyen T, Chalmers KJ (2005) Multiplex- ready markers. A technique for high throughput, low- cost genotyping on an automated ABA fragment analyzer. *In* Proceedings of the 12th Australian Annual Barley Technical Symposium, Hobart, Australia

He P, Friebe BR, Gill BS, Zhou JM (2003) Allopolyploidy alters gene expression in the highly stable hexaploid wheat. Plant Molecular Biology 52: 401- 414

Heun M, Schafer- Pregl R, Klawan D, Castagna R, Accerbi M, Borghi B, Salamini F (1997) Site of Einkorn wheat domestication identified by DNA fingerprinting. Science 278: 1312- 1314

Hole F (1998) Wheat domestication, Letter to the editor. Science 279: 303

Hu H (1996) *In vitro* produced haploids in wheat. *In* SM Jain, SK Sopory, RE Veilleux, eds, *In vitro* Haploid Production in Higher Plants, Cereals, vol 4. Kluwer academic publishers, Dordrecht, the Netherlands, p 73-97

Huang S, Sirikhachornkit A, Faris JD, Su X, Gill BS, Haselkorn R, Gornicki P (2002a). Phylogenetic analysis of the acetyl- CoA carboxylase and 3-phosphoglycerate kinase loci in wheat and other grasses. Plant Molecular Biology 48: 805-820

Huang S, Sirikhachornkit A, Su X, Faris J, Gill B, Haselkorn R, Gornicki P (2002b) Genes encoding plastid acetyl CoA carboxylase and 3- phosphoglycerate kinase of the *Triticum/ Aegilops* complex and the evolutionary history of polyploid wheat. Proceedings of the National Academy of Sciences, USA, 99 (12): 8133-8138

Huang XQ, Coster H, Ganal MW, Roder MS (2003) Advanced backcross QTL analysis for the identification of quantitative trait loci alleles from wild relatives of wheat (*Triticum aestivum* L.). Theoretical and Applied Genetics 106: 1379-1389

Huang XQ, Cloutier S, Lycar L, Radovanovic N, Humphreys DG, Noll JS, Somers DJ, Brown PD (2006) Molecular detection of QTLs for agronomic and quality traits in a doubled haploid population derived from two Canadian wheats (*Triticum aestivum* L.). Theoretical and Applied Genetics 113: 753-766

Inagaki M, Mujeeb-Kazi A(1995) Comparison of polyhaploid production frequencies in crosses of hexaploid wheat with maize, pearl millet and sorghum. Breeding Science 157-161

Ishii T, Mori N, Ogihara Y (2001) Evaluation of allelic diversity at chloroplast microsatellite loci among common wheat and its ancestral species. Theoretical and Applied Genetics 103: 896-904

Jaccoud D, Peng K, Feinstein D, Kilian A (2001) Diversity arrays: a solid state technology for sequence information independent genotyping. Nucleic Acids Research 29(4): e 25

Jamieson PD, Brooking IR, Semenov MA, McMaster GS, White JW, Porter JR (2007) Reconciling alternative models of phonological development in winter wheat. Field Crops Research 103: 36- 41

Jefferies SP, Pallotta MA, Paull JG, Karakousis A, Kretschmer JM, Manning S, Islam AKMR, Langridge P, Chalmers KJ (2000) Mapping and validation of chromosome regions conferring boron toxicity tolerance in wheat (*Triticum aestevum*). Theoretical and Applied Genetics 101: 767- 777

Jenkins S, Gibson N (2002) High- throughput SNP genotyping. Comparative and Functional Genomics 3: 57- 66

Jensen NF (1988) Plant Breeding Methodology. John Wiley & Sons, New York

John PCL, Zhang K, Dong C, Diederich L, Wightman F (1993) P34- cdc2 related proteins in control of cell cycle progression, the switch between division and differentiation in tissue development, and stimulation of division by auxin and cytokinin. Australian Journal of Plant Physiology 20: 503-526

Jones MK, Allaby RG, Brown TA (1998) Wheat domestication. Letter to the editor. Science 279: 302-303

Jordan MC, Somers DJ, Banks TW (2007) Identifying regions of the wheat genome controlling seed development by mapping expression quantitative trait loci. Plant Biotechnology Journal 5: 442-453

Kaneko M, Itoh H, Inukai Y, Sakamoto T, Ueguchi-Tanaka M, Ashikari M, Matsuoka M (2003) Where do gibberellin biosynthesis gibberellin signalling occur in rice plants? The Plant Journal 35: 104- 115

Kasha KJ, Ziauddin A, Cho UH (1990) Haploids in cereal improvement: anther and microspore culture. In JP Gustafson, ed, Gene Manipulation in Plant Improvement II, Plenum Press, New York. p. 213-235

Kato K, Yamagata H (1988) Method of evaluation of chilling requirement and narrow-sense earliness of wheat cultivars. Japanese Journal of Breeding 38: 172-186

Kerby K, Kuspira J (1988) Cytological evidence bearing on the origin of the B genome in polyploid wheats. Genome 30: 36-43

Keyes GJ, Paolillo DJ, Sorrells ME (1989) The effects of dwarfing genes *Rht1* and *Rht2* on cellular dimensions and rate of leaf elongation in wheat. Annals of Botany 64: 683- 690

Khan E M, Ellis RH, Roberts EH (1986) Effects of laboratory germination, soil temperature and moisture content on the emergence of spring wheat. Journal of Agricultural Science 107: 431-438

Kihara H (1944) Discovery of the DD-analyser one of the ancestors of *vulgare* wheats. Agriculture and Horticulture 19: 889-890

Kilian B, Ozkan H, Deusch O, Effgen S, Brandolini A, Kohl J, Martin W, Salamini F (2007) Independent wheat B and G genome origins in outcrossing Aegilops progenitor haplotypes. Molecular Biology, 24(1): 217-227

Kisana NS, Nkongolo KK, Quick JS Johnson DL (1993) Production of doubled haploids by anther culture and wheat x maize method in a wheat breeding programme. Plant Breeding 110: 96-102

Koneber DC and Boanowetz GM (1992) Phenotypic analysis of a dwarf wheat (*Triticum aestivum* L.) with altered phytochrome-mediated growth response. Plant Physiology 100: 1901-1908

Koebner R, and Summers R (2007) Marker-assisted selection in wheat: evolution, not revolution In EP Guimarães, J Ruane, BD Scherf, A Sonnino, eds, Marker Assisted Selection: Current status and future perspectives in crops, livestock, forestry and fish. Food and Agriculture Organisation of the United Nations, Rome. p. 51-58

Knox RE, Clark JM, DePauw RM (2000) Dicamba and growth condition effects on doubled haploid production in durum wheat crossed with maize. Plant Breeding119: 289- 298

Koba T, Tsunewaki K (1978) Mapping of the s and Ch 2 genes on chromosome 3D of common wheat. Wheat Information Service 45-46, 18-20

Konarev V, Gavrilyuk I, Gubareva N, Peneva T (1979) Seed proteins in genome analysis cultivar identification and documentation of cereal genetic resources: a review. Cereal Chemistry 56: 272-278

Koornneef, M., Bentsink, L., and Hilhorst, H. (2002). Seed dormancy and germination. Current Opinion in Plant Biology 5: 33-36

Korzun V, Roder MS, Ganal MW, Worland A J, Law CN (1998) Genetic analysis of the dwarfing gene (*Rht8*) in wheat. Molecular mapping of the *Rht8* on the short arm of chromosome 2D of bread wheat (*Triticum aestivum* L.). Theoretical and Applied Genetics, 96: 1104-1109

Kozub N, Xynias IN, Sozinov I, Lisova G, Zamani IA, Gouli-Vavdinoydi E, Roupakias DG (2006) Screening of high-quality bread wheat dihaploid lines by the use of biochemical markers. Russian Journal of Plant Physiology 53 (3): 396-400

Kuchel H, Fox R, Reinheimer J, Mosionek L, Willey N, Bariana H, Jefferies S (2007c) The successful application of a marker-assisted wheat breeding strategy. Molecular Breeding 20: 295-308

Kuchel H, Williams KJ, Langridge P, Eagles HA, Jefferies SP (2007a) Genetic dissection of grain yield in bread wheat. I. QTL analysis. Theoretical and Applied Genetics 115: 1029- 1041

Kuchel H, Williams KJ, Langridge P, Eagles HA, Jefferies SP (2007b) Genetic dissection of grain yield in bread wheat. II. QTL- by-environment interaction. Theoretical and Applied Genetics 115: 1015- 1027

Kuchel H, Ye G, Fox R, Jefferies S (2005) Genetic and economic analysis of a targeted marker assisted wheat breeding strategy. Molecular Breeding 16: 67-78

Kumar N, Kulwal PL, Gaur A, Tyagi AK, Khurana JP, Khurana P, Balyan H.S, Gupta PK (2006) QTL analysis for grain weight in common wheat. Euphytica 151: 135-144

Lagudah ES, McFadden H, Singh RP, Huerta-Espino J, Bariana HS, Spielmeyer W (2006) Molecular genetic characterization of the *Lr34/Yr18* slow rusting resistance gene region in wheat. Theoretical and Applied Genetics 114: 21-30

Lander ES, Boststein D (1989) Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121: 185-199

Laurie DA Bennett MD (1987) The effect of crossability loci Krl and Kr2 on fertilisation frequency in hexaploid wheat x maize crosses. Theoretical and Applied Genetics 73:403-409

Laurie DA (1989) Factors affecting fertilization frequency in crosses of *Triticum aestivum* cv. 'Highbury' x *Zea mays* cv. 'Seneca 60'. Plant Breeding 103: 133-140

Lee S, Cheng H, King KE, Wang W, He Y, Hussain A, Lo J, Harberd NP, Peng J (2002) Gibberellin regulates Arabidopsis seed germination via RGL2, a GAI/RGA-like gene whose expression is up-regulated following imbibition. Genes and Development 16: 646-658

Li WL, Nelson JC, Chu CY, Shi LH, Huang SH, Liu DJ (2002) Chromosomal locations and genetic relationships of tiller and spike characters in wheat. Euphytica 125: 357-366

Ling DX, Luckett DJ, Darvey NL (1991) Low-dose gamma irradiation promotes wheat anther culture response. Australian Journal of Botany 39:467-474

Lev-Yadun S, Gopher A, Abbo S (2000) The cradle of agriculture. Science 288: 1602-1603

Lovegrove A, Hooley R (2000) Gibberellin and abscisic acid signalling in aleurone. Trends in Plant Science 5: 102-110

Lupton FGH (1987) History of wheat breeding. *In:* FGH Lupton, ed, Wheat Breeding Its scientific basis. Chapman and Hall Ltd, London. p 31-50

Ma Z, Zhao D, Zhang C, Zhang Z, Xue S, Lin F, Kong Z, Tian D, Luo Q (2007) Molecular genetic analysis of five spike-related traits in wheat using RIL and immortalized F_2 populations. Molecular Genetics and Genomics 277: 31-42

Mac Key J (1966) Species relationship in *Triticum*. Proceedings of the 2nd International Wheat Genetics Symposium Hereditas, Suppl. 2: 237-275

Manly KF, Cudmore Jr, RH, Meer JM (2001) Map Manager QTX, cross- platform software for genetic mapping. Mammalian Genome 12: 930-932

Marsolaris A (1989) The commercial application of cereal haploidy. *In* M, Maluszynski, ed, Current options of Cereal Improvement. Doubled Haploids, Mutants and Heterosis, Kluwer Academic Publishers, the Netherlands. p 211-214

Martin JH, Waldren RP, Stamp DL (2006) *Principles of field crop production*, Ed 4. Pearson Education, Inc., Upper Saddle River, New Jersey

Matzk F, Mahn A (1994) Improved techniques for haploid production in wheat using chromosome elimination. Plant Breeding 113: 125-129

Mejza SJ, Morgant VD, Bona DE, Wong JR (1993) Plant regeneration from isolated microspores of Triticum aestivum. Plant Cell Reports 12:149-153

McFadden ES and Sears ER (1944) The artificial synthesis of *Triticum spelta*. Records of the Society for Genetics in America 13: 26-27

McFadden ES, Sears ER (1946) The origin of *Triticum spelta* and its free threshing hexaploid relatives. Journal of Heredity 37: 81-89

McGinnis KM, Thomas SG, Soule JD, Strader LC, Zale JM, Sun TP, Steber CM (2003) The *Arabidopsis* SLEEPY 1 gene encodes a putative F-box subunit of an SCF E3 ubiquitin ligase. Plant Cell 15: 1120-1130

McVittie JA, Gale MD, Marshall GA, Westcott B (1978) The intra-chromosomal mapping of the 'Norin 10' and 'Tom Thumb' dwarfing genes. Heredity 40: 67-70

Miralles DJ, Calderini DF, Pomar KP, D'Ambrogio AD (1998) Dwarfing genes and cell dimensions in different organs of wheat. Journal of Experimental Botany 49 (324): 1119-1127

Mitchell MJ, Busch RH, Rines HW (1992) Comparison of lines derived by anther culture and single-seed descent in a spring wheat cross. Crop Science 32: 1446-1451

Mohan M, Nair S, Bhagawat A, Krishna TG, Yano M, Bhatia CR, Sasaki T (1997) Genome mapping, molecular markers and marker-assisted selection in crop plants. Molecular Breeding 3: 87-103

Mok DWS, Mok MC (2001) Cytokinin metabolism and action. Annual Review of Plant physiology and Plant Molecular Biology 52: 89-118

Monte E, Amador V, Russo E, Martinez-Garcia J, Prat S (2003) PHOR1: A U-Box GA signalling component with a role in proteasome degradation? Journal of Plant Growth Regulation 22: 152-162

Morell MK, Myers AM (2005) Towards the rational design of cereal starches. Current Opinion in Plant Biology 8: 204-210

Mori N, Miyashita NT, Terachi T, Nakamura C (1997) Variation in *coxII* intron in the wild ancestral species of wheat. Hereditas 126: 281-288

Mori M, Uchino N, Chono M, Kato K, Miura H (2005) Mapping QTLs for grain dormancy on wheat chromosome 3A and the group 4 chromosomes, and their combined effect. Theoretical and Applied Genetics 110: 1315-1323

Moussavi-Nik M, Rengel Z, Pearson JN, Hollamby G (1997) Dynamics of nutrient remobilization from seed of wheat genotypes during imbibition, germination and early seedling growth. Plant and Soil 197: 271-280

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum 15: 473- 497

Nakajima M, Shimada A, Takashi Y, Kim Y, Park SH, Ueguchi-Tanaka M, Suzuki H, Katoh E, Luchi S, Kobayashi M, Maeda T, Matsuoka M, Yamaguchi I (2006) Identification and characterization of Arabidopsis gibberellin receptors. The Plant Journal 46: 880-889

Narasimhamoorthy B, Gill BS, Fritz AK, Nelson JC, Brown-Guedira GL (2006) Advanced backcross QTL analysis of a hard winter wheat x synthetic wheat population. Theoretical and Applied Genetics 112: 787-796

Naylor REL (1993) The effect of parent plant nutrition on seed size, viability and vigour and on germination of wheat and triticale at different temperatures. Annals of Applied Biology 123: 379-390

Nesbitt M, Samuel D (1998) Wheat domestication: archaeological evidence. Science 279: 1433

Nishikawa K, Furuta Y, Yamada T, Kudo S (1992) Genetic studies of α- amylase isozymes in wheat. VII. Variation in diploid ancestral species and phylogeny of tetraploid wheat. Theoretical and Applied Genetics 67: 1-15

Nyachiro JM, Clarke FR, DePauw RM, Knox RE, Armstrong KC(2002) Temperature effects on seed germination and expression of seed dormancy in wheat . Euphytica 126: 123-127

Ogbonnaya FC, Seah S, Delibes A, Jahier J, Lopez-Brana I, Eastwood RF, Lagudah ES (2001) Molecular- genetic characterization of a new nematode resistance gene in bread wheat. Theoretical and Applied Genetics 102: 623-629

Ogawa M, Hanada A, Yamauchi Y, Kuwahara A, Kamiya Y, Yamaguchi S (2003) Gibberellin biosynthesis and response during Arabidopsis seed germination. Plant Cell 15: 1591-1604

Olszewski N, Sun TP, Gubler F (2002) Gibberellin signaling: biosynthesis, catabolism, and response pathways. Plant Cell, Supplement: S61- S80.

Patnaik D**,** Khurana P **(**2001) Wheat Biotechnology: A mini review Electronic Journal of Biotechnology. (www .ejb.org/content/vo14/issue2/full/4/-)

Paolillo DJ, Sorrells ME and Keyes GJ (1991) Gibberellic acid sensitivity determines the length of the extension zone in wheat leaves. Annals of botany 67:479-485.

Pauls KP (1996) The utility of doubled haploid populations for studying the genetic control of traits determined by recessive alleles. *In:* S.Mohan Jain,S.K.Sopory, and R.E.Veilleux (eds.), *In Vitro* Haploid Production in Higher Plans. Volume 1: Fundamental Aspects and Methods, Kluwer Academic Publishers, the Nethrlands. Pp. 125-144

Peng J, Carol P, Richards DE, King KE, Cowling RJ, Murphy GP, Harberd NP (1997). The Arabidopsis GAI gene defines a signaling pathway that negatively regulates gibberellin responses. Genes and Development 11: 3194-3205

Peng J, Harberd NP (2002) The role of GA-mediated signalling in the control of seed germination. Current Opinion in Plant Biology 5: 376-381

Peng J, Richards DE, Hartley NM, Murphy GP, Devos KM, Flintham JE, Bealess J, Fish LJ, Worland AJ, Pelica F, Sudhakar D, Christou P, Snape JW, Gale MD, Harberd NP (1999) 'Green revolution' genes encode mutant gibberellin response modulators. Nature 400: 256-261

Percival J (1921) The wheat plant (A monograph). Duckworth and Co., London

Pestsova E, Ganal MW, Roder MS (2000) Isolation and mapping of microsatellite markers specific for the D genome of bread wheat. Genome 43: 689-697

Pickering RA Devaux P (1992) Haploid production: Approaches and use in plant breeding. *In* PR Shewry, ed, Barley: Genetics, Biochemistry, Molecular Biology and Biotechnology, CAB International, Wallingford, Oxon, UK Pp 519-547

Podlich DW, Christopher R, Cooper W and Cooper M (2004) Mapping as you go: An effective Approach for marker-assisted selection of complex traits Crop Science 44:1560-1571

Pratap A, Sethi GS, Chaudhary H.K (2006) Relative efficiency of anther culture and chromosome elimination techniques for haploid induction in triticale X wheat and triticale X triticale hybrids. Euphytica 150: 339-345

Provan J, Wolters P, Caldwell KH, Powell W (2004) High-resolution organellar genome analysis of *Triticum* and *Aegilops* sheds new light on cytoplasm evolution in wheat. Theoretical and Applied Genetics 108: 1182-1190

Qi LL, Echalier B, Chao S, Lazo GR, Butler GE, Anderson OD, Akhunov ED, Dvorak J, Linkiewicz AM, Ratnasiri A, Dubcovsky J, Bermudez-Kandianis CE, Greene RA, Kantety R, La Rota CM, Munkvold JD, Sorrells SF, Sorrells ME, Dilbirligi M, Sidhu D, Erayman M, Randhawa HS, Sandhu D, Bondareva SN, Gill KS, Mahmoud AA, Ma X-F, Miftahudin, Gustafson JP, Conley EJ, Nduati V, Gonzalez-Hernandez JL, Anderson JA, Peng JH, Lapitan NLV, Hossain KG, Kalavacharla V, Kianian SF, Pathan MS, Zhang DS, Nguyen HT, Choi D-W, Fenton RD, Close TJ, McGuire PE, Qualset CO, Gill BS (2004) A chromosome bin map of 16,000 expressed sequence tag loci and distribution of genes among the three genomes of polyploidy wheat. Genetics 168: 701-712

Quarrie SA, Quarrie SP, Radosevic R, Rancic D, Kaminska A, Barnes JD, Leverington M, Ceoloni C, Dodig D (2006) Dissecting a wheat QTL for yield

present in a range of environments: from the QTL to candidate genes. Journal of Experimental Botany 57 (11): 2627-2637

Rajaram S (2005) Role of Conventional Plant Breeding and Biotechnology in Future Wheat Production. Turkish Journal of Agriculture and Forestry 29: 105-111

Raman R, Raman H, Martin P (2007) Functional gene markers for polyphenol oxidase locus in bread wheat (*Triticum aestivum* L.). Molecular Breeding 19 (4): 315-328

Rao MVP (1977) Mapping of the sphaerococcum gene 's' on chromosome 3D of wheat. Cereal Research Communication 5: 15-17

Rao MVP (1981) Telocentric mapping of the Arm Inhibitor gene Hd on chromosome 4B of common wheat. Cereal Research Communication 5: 15-17

Riera-Lizarazu O and Mujeeb-Kazi A (1993) Polyhaploid Production in the Triticeae: Wheat x Tripsacum Crosses. Crop Science 33:973-976

Reynolds MP, Borlaug NE (2006) Impacts of breeding on international collaborative wheat improvement. Journal of Agricultural Science 144: 3-17.

Roder MS, Korzum V, Gill BS, Ganal MW (1998) The physical mapping of microsatellite markers in wheat. Genome 41: 278-283

Roder MS, Huang X, Borner A (2008) Fine mapping of the region on wheat chromosome 7D controlling grain weight. Functional & Integrative Genomics 8: 79-86

Roses AD (2002) Pharmacogenetics place in modern medical science and practice. Life Science 70: 1471-1480

Salamini F, Ozkan H, Brandolini A, Schafer- Pregl R, Martin W (2002) Genetics and geography of wild cereal domestication in the near east. Nature 3: 429- 441

Salina E, Borner A, Leonova I, Korzun V, Laikova L, Maystrenko O, Roder MS (2000) Microsatellite mapping of the induced sphaerococcoid mutation genes in *Triticum aestivum*. Theoretical and Applied Genetics 100: 686-689

Salina EA, Leonova IN, Roder MS, Laikova LI, Maystrenko OI, Budashkina EB, Shumnyi VK (2001) Russian Journal of Plant Physiology 48 (3): 377-381

Sarkar P, Stebbins GL (1956) Morphological evidence concerning the origin of the B genome of wheat. American Journal of Botany 43: 297-304

Sarrafi A, Amrani N, Alibert G (1994) Haploid regeneration from tetraploid wheat using maize pollen. Genome 37: 176-178

SAS (1990) *SAS/STAT* user's guide, Version 6, Ed 4, Vol 1. Cary, NC: SAS Institute Inc.

Sasaki A, Itoh H, Gomi K, Ueguchi-Tanaka M, Ishiyama K, Kobayashi M, Jeong D, An G, Kitano H, Ashikari M, and Matsuoka M (2003) Accumulation of Phosphorylated Repressor for Gibberellin Signaling in an F-box Mutant. Science 299 (5114): 1896– 898

Sauter M, Kende H (1992) Gibberellin-induced growth and regulation of the cell division cycle in deepwater rice. Planta 188: 362–368

Sears ER (1944) The artificial synthesis of *Triticum spelta* Records of Genetic Society of America 13: 26-27

Sears ER (1946) Isochromosomes and telocentrics in *Triticum vulgare*. Genetics 31: 229–230

Sears ER (1947) The *sphaerococcum* gene in wheat. Genetics, 32:102-103

Sears ER (1954) The aneuploids of common wheat, Missouri Agriculture Experiment Station research bulletin 572: 1-58

See DR, Brooks S, Nelson JC, Brown-Guedira G and Gill BS (2006) Gene evolution at the ends of wheat chromosomes Proceedings of the National Academy of Sciences, USA 103: 4162-4167

Schmidt JW, Dale EW, Johnson VA (1963) Inheritance of an incompletely dominant character in common wheat simulating *Triticum sphaerococcum*. Crop Science 3: 261-264

Schmidt JW, Johnson VA (1963) A *sphaerococcum*- like tetraploid wheat. Crop Science 3: 98-99

Schmidt JW, Johnson VA (1966) Inheritance of the *sphaerococcum* effect in tetraploid wheat. Wheat Information Service 22: 5

Schuelke M (2000) An economic method for the fluorescent labelling of PCR fragments. A poor man's approach to genotyping for research and high-throughput diagnostics. Nature Biotechnology 18: 233-234

Semagn K, Bjornstad A, Skinnes H, Maroy AG, Tarkegne Y, William M (2006) Distribution of DarT, AFLP, and SSR markers in a genetic linkage map of a doubled- haploid hexaploid wheat population. Genome 49: 545-555

Shewry PR, Halford NG (2002) Cereal seed storage proteins: structures, properties, and role in grain utilization. Journal of Experimental Botany 53: 947-958

Shimada A, Ueguchi-Tanaka M, Sakamoto T, Fujioka S, Takatsuto S, Yoshida S, Sazuka T, Ashikari M, Matsuoka M (2006) The rice *SPINDLY* gene functions as a negative regulator of gibberellin signaling by controlling the suppressive function of the DELLA protein, SLR1, and modulating brassinosteroid synthesis. The Plant Journal 48: 390–402

Shindo C, Tsujimoto H, Sasakuma T (2003) Segregation analysis of heading traits in hexaploid wheat utilizing recombinant inbred lines. Heredity 90: 56-63.

Sibi ML, Kobaissi A, Shekafandeh A (2001) Green haploid plants from unpollinated ovary culture in tetraploid wheat (Triticum durum Defs.). Euphytica 22: 351-359.

Simmons KJ, Fellers JP, Trick HN, Zhang Z, Tai YS, Gill BS, Faris JD (2006) Molecular characterization of the major wheat domestication gene Q. Genetics 172: 547-555.

Simonetti MC, Bellomo MP, Laghetti G, Perrino P, Simeone R and Blanco A (1999) Quantitative trait loci influencing free-threshing habit in tetraploid wheats. Genetic Resources and Crop Evolution 46: 267-271.

Singh D (1987) Mapping of the complex *T. sphaerococcum* locus. Wheat Information Service 64: 17-20

Singh RD (1946) *Triticum sphaerococcum* Perc. (Indian dwarf wheat). The Indian Journal of Genetics and Plant Breeding 6: 34-47.

Singh RP, Rajaram S, Miranda A, Hureta-Espino J, Autrique E (1998) Comparison of two crossing and four selection schemes for yield, yield traits and slow rusting resistance to leaf rust in wheat. Euphytica 100: 35-43.

Slafer GA, Rawson HM (1994) Sensitivity of wheat phasic development to major environmental factors: A re-examination of some assumptions made by physiologists and modellers. Australian Journal of Plant Physiology 21: 393-426.

Sleper DA, Poehlman JM (2006) Breeding Field Crops, ed.5. Blackwell Publishing, Oxford, UK.

Smith BD (2001) Documenting plant domestication: The consilience of biological and archaeological approaches. Proceedings of the National Academy of Sciences, USA 98: 1324-1326

Snape J.W, Simpson E, Parker BB, Friedt W, Fonghi-Wehr B (1986) Criteria for the selection and use of double haploid systems in cereal breeding programmes.

In: W Horn, CJ Jensen, W Odenbach , O Schieder, Eds, *Genetic Manipulation in Plant Breeding*. Proceedings of the International Symposium, Eucarpia, W.de Gruyter, Berlin, p 217-229

Snape JW, Parker BB (1987) Comparison of methods of producing doubled haploids in wheat and barley. Annual Report of the Plant Breeding Institute, Cambridge, UK, p 72-73

Snape JW, Ouyang JW, Parker BB, Jia SE (1992) Evidence for genotypic selection in wheat during the development of recombinant inbred lines by anther culture and single seed descent. Journal of Genetics and Breeding 46:167-172

Somers DJ, Issac P, Edwards K (2004) A high density microsatellite consensus map for bread wheat (*Triticum aestivum* L.). Theoretical and Applied Genetics 109: 1105-1114

Somers DJ, Fedak G, Savard M (2003a) Molecular mapping of novel genes controlling Fusarium head blight resistance and deoxynivalenol accumulation in spring wheat. Genome 46: 555-564

Somers DJ, Kirkpatrick R, Moniwa M, Walsh A (2003b) Mining single nucleotide polymorphisms from hexaploid wheat ESTs. Genome 46: 431- 437

Song QJ, Shi JR, Sing S, Fickus EW, Costa JM, Lewis, Gill BS, Ward R, Cregan PB (2005) Development and mapping of microsatellite (SSR) markers in wheat. Theoretical and Applied Genetics 110: 550-560

Sourdille P, Cadalen T, Gay G, Gill B, Bernard M (2002) Molecular and physical mapping of genes affecting awning in wheat. Plant breeding: 121: 320-324

Spielmeyer W, Hyles J, Joaquim P, Azanza F, Bonnett D, Ellis ME, Moore C, Richards RA (2007) A QTL on chromosome 6A in bread wheat (*Triticum aestivum*) is associated with longer coleoptiles, greater seedling vigour and final plant height. Theoretical and Applied Genetics 115: 59-66

Stoddard FL (1999) Variation in grain mass, grain nitrogen, and starch B- granule content within wheat heads. Cereal Chemistry 76: 139-144

Suenaga K, Morshedi AR, Darvey NL (1997) Haploid production of Australian wheat (*Triticum aestivum* L.) cultivars through wheat x maize (*Zea mays* L.) cross. Australian Journal of Agricultural Research 48: 1207-1211

Suenaga K, Singh RP, Huerta-Espino J, William HM (2003) Microsatellite markers for genes Lr34/Yr 18 and other quantitative trait loci for leaf rust and stripe rust resistance in bread wheat. Phytopathology 93: 881-890

Suenaga K, Khairallah M, William HM, Hoisington DA (2005) A new intervarietal linkage map and its application for quantitative trait locus analysis of 'gigas' features in bread wheat. Genome 48: 65-75

Sullivan JA, Shirasu K, Deng X-W, (2003) The diverse role of ubiquitin and the 26S proteasome in the life of plants. Nature Review of Genetics 4: 948-958

Sun T, Gubler F (2004) Molecular mechanism of gibberellin signalling in plants. Plant Biology 55:197-223

Sun XY, Wu K, Zhao Y, Kong FM, Han GZ, Jiang HM, Huang XJ, Li RJ, Wang HG, Li SS (2008) QTL analysis of kernel shape and weight using recombinant inbred lines in wheat. Euphytica (Online) 1573-5060

Swaminathan MS (1965) The origin of macro-from micro-mutations and factors governing the direction of micro-mutational changes. Proceedings of the 3rd International Symposium. The impact of Mendelism on agriculture Biology and Medicine. Indian Journal of Genetics and Plant Breeding. 26A: 29-41

Swaminathan MS (2006) An evergreen revolution. Crop Science 46: 2293-2303

Swaminathan MS, Jagathesan D, Chopra VL (1963) Induced sphaerococcoid mutations in *Triticum aestivum* and their phylogenetic and breeding significance. Indian Journal of Genetics and Plant Breeding 539-540

Swain SM, Sing DP (2005) Tall tales from sly dwarves: Novel functions of gibberellins in plant development. Trends in Plant Science 10: 123-129

Talbert LE, Smith LY, Blake NK (1998) More than one origin of hexaploid wheat is indicated by sequence comparison of low copy DNA. Genome 41: 402-407

Tanno K, Willcox G (2006) How fast was wild wheat domesticated? Science 288: 1604

Thomas SG, Sun T (2004) Update on gibberellin signaling. A tale of the tall and the short. Plant Physiology 135: 668-676

Tonkinson CL, Lyndon RF, Arnold GM, Lenton JR (1995) Effect of the *Rht3* dwarfing gene on dynamics of cell extension in wheat leaves, and its modification by gibberellic acid and paclobutrazol. Journal of Experimental Botany 46**:** 1085–1092

Triboi E, Martre P, Triboi-Blondel AM (2003) Envronmentally- induced changes in protein composition in developing grains of wheat are related to changes in total protein content. Journal of Experimental Botany 54: 1731-1742

Tsuji H, Aya K, Ueguchi-Tanaka M, Shimada Y, Nakazono M, Watanabe R, Ito H, Nishizawa N, Gomi K, Shimada A, Kitano H, Ashikari M, Atsuoka M (2006). GAMYB controls different sets of genes and is differentially regulated by microRNA in aleurone cells and anthers. Plant Journal 47, 427–444

Tsunewaki K, Takumi S, Mori N, Achiwa T, Liu YG (1991) Origin of polyploid wheats revealed by RFLP analyses.In: Sasakuma T, Kinoshita T eds, Nuclear and organellar genomes of wheatspecies Yokohima, Japan: Kihara Memorial Foundation, p 9-31

Tyler L, Thomas SG, Hu J, Dill A, Alonso JM, Ecker JR, Sun T (2004) DELLA Proteins and gibberellin-regulated seed germination and floral development in Arabidopsis. Plant Physiology 135: 1008-1019

Ueguchi-Tanaka M., Motoyuki A, Nakajima M, Itoh H, Katoh E, Kobayashi M, Chow T, Hsing YC, Kitano H, Yamaguchi I, Matsuoka M (2005) Gibberellin insensitive dwarf 1 encodes a soluble receptor for gibberellin. Nature 437: 693-697

Ueguchi-Tanaka M, Nakajima M, Motoyuki A, Matsuoka M (2007) Gibberellin receptor and its role in gibberellin signalling in plants. Plant Biology 58: 183-198

Ushiyama T, Kuwabara T, Yoshida T (2007) Effects of various phytohormones on haploid wheat production in wheat x maize crosses. Plant Production Science 10(1): 36-41

Verma V, Worland AJ, Sayers EJ, Fish L, Caligari PDS, Snape, JW (2005) Identification and characterization of quantitative trait loci related to lodging resistance and associated traits in bread wheat. Plant Breeding 124: 234-241

Vierstra RD (2003) The ubiquitin/26S proteasome pathway, the complex last. The life of many plant proteins. Trends Plant Science 8: 135-142

Vriezen WH, Achard P, Harberd NP, Van Der Straeten D (2004) Ethylene-mediated enhancement of apical hook formation in etiolated Arabidopsis thaliana seedlings is gibberellin dependent. The Plant Journal 37: 506-516

Wenzl P, Carling J, Kudrna D, Jaccoud D, Huttner E, Kleinhofs A, Kilian A (2004) Diversity arrays technology (DArT) for whole-genome profiling of barley. Proceedings of the National Academy of the United States of America 101(26), 9915-9920

Weyhrich RA, Carver BF, Smith EL (1994) Effects of awn suppression on grain yield and agronomic traits in hard red winter wheat. Crop Science 34: 965-969

William HM, Trethowan R, Crosby-Galvan EM (2007) Wheat breeding assisted by markers: CIMMYT's experience. Euphytica 157: 307-319

Wilson ID, Barker GLA, Chungui L, Coghill JA, Beswick RW, Lenton JR, Edwards KJ (2005) Alteration of the embryo transcriptome of hexaploid winter wheat (*Triticum aestivum* cv. Mercia) during maturation and germination. Functional and Integrated Genomics 5: 144-154

Worland AJ, Sayers EJ, Korzun V (2001) Allelic variation at the dwarfing gene *Rht8* locus and its significance in international breeding programmes. Euphytica 119: 155-159

Yamaguchi S, Kamiya Y (2000) Gibberellin biosynthesis: its regulation by endogenous and environmental signals. Plant Cell Physiology 41: 251-257

Yang S, Yu H, Xu Y, Goh CJ (2003) Investigation of cytokinin-deficient phenotypes in Arabidopsis by ectopic expression of orchid DSCKX1. Federation of European Biochemical Societies Letters 555: 291-296

Young ND (1999) A cautiously optimistic vision for marker- assisted breeding. Molecular Breeding 5: 505-510

Youssefian S, Kirby EJM, Gale MD (1992a) Pleiotropic effects of the GA-insensitive Rht dwarfing genes in wheat.1.Effects on development of the ear, stem and leaves. Field Crops Research 28, 179-190

Youssefian S,.Kirby EJM, Gale MD (1992b) Pleiotropic effects of the GA-insensitive *Rht* dwarfing genes in wheat. Effects on leaf, stem, ear and floret growth. Field Crops Research 28, 191-210

Zeven AC (1980) The spread of bread wheat over the old world since the neolithicum as indicated by its genotype for hybrid necrosis. Journal de l'Agriculture Tropicale et Botanique Appliquée 27:19-53

Zhang J, Friebe N, RauppWJ, Harrison SA, Gill BS (1996) Wheat embryogenesis and haploid production in wheat z maize hybrids. Euphytica 90:315-324

Zhang K, Letham DS, John PCL (1996) Cytokinin controls the cell cycle at mitosis by stimulating the tyrosine dephosphorylation and activation of p34cdc2-like H1 histone kinase. Planta 200: 2-12

Zhang W, Qu LJ, Gu H, Gao W, Liu M, Chen J, Chen Z (2002) Studies on the origin and evolution of tetraploid wheats based on the internal transcribed spacer

(ITS) sequences of nuclear ribosomal DNA. Theoretical and applied Genetics 104: 1099-1106

Zhang X, Yang S, Zhou Y, He Z, Xia X (2006) Distribution of the *Rht-B1b*, *Rht-D1b* and *Rht8* reduced height genes in autumn-sown Chinese wheats detected by molecular markers. Euphytica 152: 109-116

Zheng MY, Liu W, Weng Y, Polle E, Konzak CF (2001) Culture of freshly isolated wheat (*Triticum aestivum* L.) microspores treated with inducer chemicals. Plant Cell 20: 685-690

APPENDIX I: LIST OF THE DH LINES USED TO CREATE Fig. 3.14

Effect of 10 mg l^{-1} GA_3 on the growth of DH lines

Significantly Different		Not Significantly Different	
2	73	1	74
3	75	5	77
4	76	7	82
6	78	8	84
9	79	14	85
10	80	15	86
11	81	16	89
12	83	17	90
13	87	20	92
18	88	21	94
19	91	23	95
22	93	26	97
24	96	28	98
25	99	32	101
27	100	33	103
29	102	36	104
30	106	37	105
31	109	38	107
34	110	40	108
35	113	41	111
39	115	44	112
42	116	46	114
43	117	47	120
45	118	49	121
48	119	52	125
50	122	53	126
51	123	54	129
56	124	55	133
57	127	58	134
60	128	59	135
61	130	63	137
62	131	66	
64	132	67	
65	136	68	
71	138	69	
72	139	70	
	140		

APPENDIX II- DOUBLED HAPLOID GRAIN MEASUREMENTS DATA

DH#	Whole Grain (Average)			Embyro (Average)		Grain Parameters (Average)				Seed type
	Length (mm)	Width (mm)	Depth (mm)	Length (mm)	Width (mm)	Grain L/W	Embryo L/W	% Out Crease	% Inn Crease	
1	5.04	4.03	3.39	2.57	2.12	1.26	1.23	26.43	52.37	SP5
2	4.87	3.26	3.11	2.31	1.77	1.50	1.32	22.20	46.01	SP5
3	6.94	3.39	3.04	2.82	1.78	2.05	1.63	21.23	49.04	Otane
4	6.70	3.48	3.08	2.71	1.80	1.94	1.52	21.58	48.91	Otane
5	6.62	3.55	2.98	2.88	1.87	1.87	1.58	24.50	55.83	Otane
6	7.01	3.54	3.20	2.81	1.78	1.99	1.59	20.23	58.85	Otane
7	6.84	3.42	2.77	2.58	1.56	2.01	1.67	24.74	60.87	Otane
8	6.24	3.76	3.18	2.73	1.68	1.66	1.63	23.48	55.85	Otane-SP5
9	6.65	3.62	3.18	2.76	1.73	1.84	1.61	21.76	56.92	Otane
10	7.06	3.85	3.35	2.82	1.80	1.84	1.59	26.02	52.05	Otane-SP5
11	6.57	3.69	3.19	2.67	1.64	1.78	1.65	20.33	52.11	Otane
12	5.02	3.74	3.36	2.35	1.68	1.35	1.41	27.03	41.87	SP5
13	6.72	3.55	3.13	2.55	1.67	1.90	1.53	23.51	51.25	Otane
14	5.10	3.50	3.17	2.33	1.74	1.46	1.36	18.78	46.34	SP5
15	6.75	3.64	3.07	2.56	1.58	1.87	1.64	20.59	49.62	Otane
16	5.37	3.59	3.18	2.28	1.69	1.50	1.37	20.65	40.34	Otane-SP5
17	6.46	3.46	2.89	2.50	1.38	1.87	1.84	20.75	57.23	Otane
18	6.44	3.29	2.84	2.50	1.33	1.97	1.89	25.28	54.42	Otane
19	4.83	3.52	3.56	2.18	1.45	1.38	1.52	21.04	42.52	SP5
20	6.34	3.44	2.99	2.59	1.45	1.85	1.80	26.10	52.57	Otane
21	5.15	3.75	3.54	2.31	1.50	1.38	1.55	22.87	41.13	SP5
22	5.98	3.35	2.79	2.32	1.45	1.80	1.62	29.29	52.81	Otane-SP5
23	7.05	3.33	2.89	2.61	1.53	2.12	1.72	26.51	53.73	Otane
24	6.33	3.52	2.94	2.57	1.49	1.81	1.74	27.77	47.31	Otane
25	4.97	3.81	3.29	2.23	1.42	1.31	1.60	21.64	33.17	SP5
26	6.14	3.57	3.13	2.51	1.58	1.73	1.62	23.42	50.05	Otane

27	6.10	3.57	3.12	2.50	1.57	1.72	1.62	23.58	49.61	Otane
28	5.06	3.46	3.16	2.34	1.47	1.47	1.60	19.41	39.67	SP5
30	7.03	3.67	3.32	2.78	1.52	1.92	1.83	21.83	47.30	Otane
31	5.97	3.55	3.13	2.46	1.53	1.69	1.63	23.18	47.53	SP5
32	6.97	3.65	3.18	2.62	1.46	1.91	1.81	28.73	53.03	Otane
33	6.04	3.54	3.12	2.46	1.52	1.72	1.65	23.42	47.87	SP5
34	5.02	3.55	3.25	2.15	1.42	1.42	1.54	24.89	34.84	SP5
35	6.59	3.72	3.22	2.56	1.52	1.78	1.71	21.12	42.71	Otane
36	4.88	3.36	3.31	2.27	1.40	1.46	1.63	24.51	34.30	SP5
37	5.97	3.53	3.14	2.45	1.47	1.70	1.68	23.96	46.41	SP5
38	5.94	3.54	3.15	2.44	1.48	1.69	1.67	24.13	45.84	Otane
39	6.62	3.46	2.84	2.44	1.35	1.92	1.82	21.88	49.66	Otane
40	6.62	3.27	2.80	2.33	1.36	2.04	1.72	22.86	66.11	Otane
41	5.16	3.70	3.35	2.36	1.49	1.41	1.59	18.09	41.50	SP5
42	6.19	3.30	2.99	2.51	1.30	1.88	1.94	20.97	48.35	Otane
43	6.25	3.04	2.73	2.48	1.37	2.06	1.83	23.25	52.26	Otane
44	6.65	3.44	2.96	2.42	1.39	1.94	1.75	22.86	53.38	Otane
45	4.98	3.46	3.31	2.26	1.52	1.45	1.50	21.21	40.68	SP5
46	5.04	3.30	3.06	2.43	1.50	1.53	1.64	21.56	44.22	SP5
47	6.56	3.46	2.98	2.67	1.53	1.90	1.74	23.24	51.38	Otane
48	6.42	3.53	2.91	2.57	1.41	1.83	1.83	19.47	46.50	Otane
49	6.22	2.86	2.55	2.37	1.20	2.19	1.99	27.29	50.41	Otane
50	4.85	3.63	3.30	2.18	1.47	1.34	1.49	19.87	38.26	SP5
51	5.07	3.79	3.33	2.30	1.63	1.34	1.42	23.52	41.65	SP5
52	5.12	3.68	3.40	2.18	1.48	1.39	1.48	23.20	38.34	SP5
53	4.87	3.52	3.21	2.25	1.47	1.39	1.55	26.56	38.32	SP5
54	6.67	3.17	3.01	2.68	1.47	2.12	1.84	26.69	55.58	Otane
55	4.82	3.70	3.23	2.23	1.48	1.30	1.51	18.42	41.01	SP5
56	6.56	3.70	3.02	2.54	1.49	1.78	1.71	21.82	51.85	Otane
57	5.07	3.50	3.43	2.33	1.57	1.46	1.50	22.92	39.59	SP5
58	6.64	3.63	3.31	2.72	1.60	1.83	1.71	23.92	53.12	Otane

59	6.09	3.36	3.00	2.59	1.55	1.82	1.70	21.60	46.65	Otane
60	6.78	3.58	3.05	2.65	1.52	1.90	1.75	20.07	51.04	Otane
61	6.62	3.65	3.15	2.64	1.64	1.82	1.62	19.89	52.37	Otane
62	6.90	3.58	2.99	2.56	1.41	1.93	1.83	26.61	60.70	Otane
63	6.74	3.77	3.02	2.61	1.43	1.79	1.83	23.02	56.69	Otane
64	6.92	3.65	3.28	2.93	1.67	1.90	1.76	21.38	52.44	Otane
65	4.75	3.67	3.54	2.41	1.76	1.30	1.38	25.28	43.85	SP5
66	4.63	3.15	3.03	2.16	1.41	1.48	1.54	30.63	36.88	SP5
67	6.42	3.46	2.94	2.70	1.46	1.86	1.87	25.24	57.37	Otane
68	5.11	3.66	3.25	2.40	1.59	1.40	1.52	26.03	37.41	SP5
70	6.82	3.45	2.91	2.47	1.33	1.98	1.87	26.17	54.15	Otane
71	6.68	3.53	3.02	2.55	1.45	1.90	1.77	19.93	55.39	Otane
72	6.57	3.60	3.09	2.67	1.51	1.83	1.78	19.10	54.11	Otane
73	5.16	3.71	3.52	2.34	1.65	1.40	1.42	17.48	43.15	SP5
74	6.50	3.57	3.41	2.82	1.58	1.82	1.78	18.29	50.77	Otane
75	6.71	3.40	3.03	2.57	1.38	1.98	1.88	17.04	54.00	Otane
76	6.73	3.26	2.70	2.54	1.38	2.08	1.85	29.77	53.82	Otane
77	6.41	3.56	3.18	2.65	1.49	1.80	1.79	22.67	56.37	Otane
78	6.24	3.68	3.16	2.80	1.50	1.70	1.87	22.79	54.88	Otane
79	5.03	3.56	3.31	2.39	1.57	1.42	1.53	21.68	46.49	SP5
81	6.88	3.53	3.03	2.68	1.49	1.95	1.81	20.75	55.81	Otane
82	4.98	3.42	3.48	2.10	1.47	1.46	1.44	21.45	38.48	SP5
83	6.74	3.71	3.24	2.87	1.55	1.82	1.87	22.06	53.08	Otane
84	6.38	3.59	3.12	2.61	1.52	1.78	1.74	22.47	54.98	Otane
85	5.17	3.39	3.21	2.29	1.57	1.53	1.46	23.61	46.02	SP5
86	5.38	3.50	3.36	2.45	1.66	1.54	1.48	19.47	42.93	SP5
87	4.76	3.43	3.28	2.17	1.55	1.39	1.41	19.96	37.82	SP5
88	6.51	3.68	3.11	2.65	1.57	1.77	1.70	19.95	52.05	Otane
89	6.65	3.30	2.94	2.69	1.52	2.02	1.78	22.31	56.02	Otane
90	4.97	3.62	3.41	2.37	1.75	1.38	1.36	21.30	35.85	SP5
91	5.09	3.48	3.36	2.30	1.56	1.47	1.48	19.40	36.50	SP5

92	4.91	3.59	3.28	2.32	1.63	1.37	1.44	21.92	39.02	SP5
93	6.38	3.39	2.80	2.56	1.51	1.88	1.71	19.83	51.91	Otane
94	4.94	3.72	3.33	2.35	1.69	1.33	1.39	15.39	45.61	SP5
95	6.75	3.45	3.01	2.62	1.51	1.96	1.75	18.89	58.20	Otane
96	6.00	3.34	2.88	2.49	1.47	1.80	1.71	21.30	57.76	Otane
97	6.42	3.43	3.02	2.44	1.45	1.88	1.69	20.27	53.76	Otane
98	6.39	3.17	2.69	2.48	1.32	2.03	1.89	23.49	56.92	Otane
99	6.11	3.39	2.98	2.62	1.49	1.81	1.77	23.72	53.55	Otane
100	6.37	3.45	3.06	2.63	1.53	1.85	1.73	20.28	56.05	Otane
101	6.42	3.67	3.19	2.65	1.58	1.75	1.69	19.60	50.97	Otane
102	6.49	3.70	3.28	2.77	1.65	1.75	1.69	21.74	46.69	Otane
103	6.84	3.55	3.17	2.66	1.54	1.93	1.74	19.53	54.40	Otane
104	6.68	3.53	3.05	2.80	1.64	1.89	1.72	22.28	55.83	Otane
105	6.59	3.55	3.04	2.75	1.51	1.86	1.83	20.60	55.86	Otane
106	6.78	3.72	3.21	2.78	1.58	1.83	1.77	20.57	56.28	Otane
107	4.98	3.60	3.59	2.43	1.71	1.39	1.43	26.32	42.98	SP5
108	7.18	3.23	2.91	2.77	1.44	2.23	1.94	21.38	54.75	Otane
109	6.93	3.53	3.07	2.63	1.49	1.97	1.78	20.26	50.71	Otane
110	6.95	3.55	3.08	2.73	1.58	1.96	1.74	23.70	60.66	Otane
112	6.63	3.56	3.01	2.70	1.44	1.87	1.88	26.65	57.01	Otane
113	6.14	3.32	2.88	2.45	1.33	1.85	1.85	25.45	52.69	
115	5.09	3.58	3.39	2.27	1.54	1.42	1.49	22.05	42.27	SP5
116	6.47	3.40	2.88	2.61	1.53	1.91	1.72	22.63	55.53	Otane
117	5.15	3.52	3.16	2.33	1.55	1.46	1.51	25.42	37.04	SP5
118	5.09	3.36	3.19	2.29	1.56	1.52	1.47	23.49	44.88	SP5
119	6.60	3.30	2.80	2.78	1.48	2.01	1.89	22.93	56.57	Otane
120	4.74	3.52	3.36	2.33	1.61	1.35	1.46	20.99	40.88	SP5
121	4.72	3.57	3.40	2.31	1.62	1.33	1.43	22.00	37.83	SP5
122	6.84	3.62	3.14	2.81	1.64	1.90	1.73	21.21	59.67	Otane
123	6.73	3.58	2.96	2.78	1.69	1.89	1.66	22.03	51.06	Otane
124	6.91	3.43	2.94	2.60	1.55	2.02	1.69	26.93	55.62	Otane

125	4.56	3.18	3.33	2.22	1.55	1.44	1.44	24.75	37.93	SP5
126	5.05	3.68	3.59	2.52	1.78	1.38	1.43	20.01	48.31	SP5
127	6.34	3.33	2.96	2.49	1.43	1.90	1.75	21.67	55.14	Otane
128	6.48	3.32	2.81	2.53	1.48	1.96	1.72	20.90	54.73	Otane
130	5.01	3.58	3.43	2.22	1.55	1.40	1.44	25.70	21.13	SP5
131	6.35	3.42	2.87	2.50	1.39	1.86	1.80	22.82	55.15	Otane
132	6.35	3.39	3.05	2.67	1.59	1.88	1.70	23.51	51.75	Otane
133	6.94	3.47	2.97	2.72	1.52	2.00	1.80	24.06	53.12	Otane
134	5.00	3.36	2.91	2.18	1.37	1.49	1.61	22.81	47.50	SP5
135	4.86	3.41	3.33	2.32	1.56	1.43	1.50	20.95	45.78	SP5
136	6.82	3.70	3.28	2.90	1.69	1.85	1.73	23.26	59.68	Otane
137	6.81	3.44	3.06	2.80	1.55	1.98	1.82	23.10	58.88	Otane
138	5.00	3.59	3.31	2.39	1.64	1.40	1.46	21.28	49.22	SP5
139	5.25	3.74	3.45	2.52	1.68	1.41	1.50	19.27	47.56	SP5
140	6.90	3.56	3.14	2.74	1.53	1.94	1.79	23.02	60.35	Otane
141	6.32	3.53	3.23	2.83	1.70	1.80	1.68	21.79	54.96	Otane
SP5	5.10	3.76	3.29	2.33	1.68	1.36	1.41	26.22	43.14	SP5
Ot	6.78	3.54	2.97	2.65	1.55	1.92	1.72	24.13	52.71	Otane

APPENDIX III: PHENOTYPIC TRAITS

Plant ID	Gr_Shape	PlantHt_cm	AwnLth_mm	HeadHt_mm	GrainL/W	EmbryoL/W	Out_Crease	Inn_Crease
Otane	1	115	78	91	1.92	1.72	24.13	52.71
Sp5	2	99	24	50	1.36	1.41	26.22	43.14
DH1	2	49	49	32.25	1.26	1.23	26.43	52.37
DH2	2	66.27	66.27	44.67	1.5	1.32	22.2	46.01
DH3	1	77.4	77.4	74.5	2.05	1.63	21.23	49.04
DH4	1	71.1	71.1	76.5	1.94	1.52	21.58	48.91
DH5	1	69.15	69.15	57.5	1.87	1.58	24.5	55.83
DH6	1	106.95	106.95	70.5	1.99	1.59	20.23	58.85
DH7	1	86.13	86.13	77.75	2.01	1.67	24.74	60.87
DH8	1	73.65	73.65	63.25	1.66	1.63	23.48	55.85
DH9	1	82	82	61	1.84	1.61	21.76	56.92
DH10	1	65.05	65.05	61.75	1.84	1.59	26.02	52.05
DH11	1	80.8	80.8	66.75	1.78	1.65	20.33	52.11
DH12	2	80.35	80.35	52.5	1.35	1.41	27.03	41.87
DH13	1	107.73	107.73	73	1.9	1.53	23.51	51.25
DH14	2	44.43	44.43	35	1.46	1.36	18.78	46.34
DH15	1	89.93	89.93	49.25	1.87	1.64	20.59	49.62
DH16	2	51.23	51.23	42.75	1.5	1.37	20.65	40.34
DH17	1	104.2	104.2	64.33	1.87	1.84	20.75	57.23
DH18	1	89.68	89.68	70	1.97	1.89	25.28	54.42
DH19	2	64.53	64.53	29.75	1.38	1.52	21.04	42.52
DH20	1	59.7	59.7	60.5	1.85	1.8	26.1	52.57
DH21	2	89.68	89.68	43.5	1.38	1.55	22.87	41.13
DH22	1	85.83	85.83	64	1.8	1.62	29.29	52.81
DH23	1	109.1	109.1	78.5	2.12	1.72	26.51	53.73
DH24	1	117.3	117.3	78.25	1.81	1.74	27.77	47.31
DH25	2	84.28	84.28	43	1.31	1.6	21.64	33.17
DH26	1	123.43	123.43	76.67	2.24	1.93	27.67	48.81
DH27	1	110.15	110.15	68.75	1.96	1.82	23.77	47.13
DH28	2	40.9	40.9	35.67	1.47	1.6	19.41	39.67

DH30	1	82.15	82.15	73	1.92	1.83	21.83	47.3
DH31	2	86.65	86.65	60	1.49	1.58	21.52	33.69
DH32	1	91.95	91.95	77.25	1.91	1.81	28.73	53.03
DH33	2	31.2	31.2	30.75	1.51	1.7	21.81	42.08
DH34	2	96.25	96.25	50	1.42	1.54	24.89	34.84
DH35	1	105.68	105.68	72.5	1.78	1.71	21.12	42.71
DH36	2	42.4	42.4	28	1.46	1.63	24.51	34.3
DH37	2	51.57	51.57	29	1.33	1.62	29.24	32.48
DH38	1	93.93	93.93	49	1.86	1.77	22.79	47.93
DH39	1	105.1	105.1	61.25	1.92	1.82	21.88	49.66
DH40	1	73.93	73.93	26	2.04	1.72	22.86	66.11
DH41	2	49.55	49.55	42	1.41	1.59	18.09	41.5
DH42	1	113.98	113.98	75.25	1.88	1.94	20.97	48.35
DH43	1	114.73	114.73	71.5	2.06	1.83	23.25	52.26
DH44	1	95.55	95.55	71	1.94	1.75	22.86	53.38
DH45	2	91.15	91.15	47.5	1.45	1.5	21.21	40.68
DH46	2	29.37	29.37	19.33	1.53	1.64	21.56	44.22
DH47	1	80.95	80.95	62.75	1.9	1.74	23.24	51.38
DH48	1	88.2	88.2	77.75	1.83	1.83	19.47	46.5
DH49	1	80.53	80.53	69.25	2.19	1.99	27.29	50.41
DH50	2	67.9	67.9	31	1.34	1.49	19.87	38.26
DH51	2	66.45	66.45	39.5	1.34	1.42	23.52	41.65
DH52	2	49.33	49.33	34.33	1.39	1.48	23.2	38.34
DH53	2	41.58	41.58	32.25	1.39	1.55	26.56	38.32
DH54	1	82.15	82.15	69	2.12	1.84	26.69	55.58
DH55	2	43.27	43.27	29	1.3	1.51	18.42	41.01
DH56	1	95.63	95.63	80.67	1.78	1.71	21.82	51.85
DH57	2	64.9	64.9	30.33	1.46	1.5	22.92	39.59
DH58	1	112.8	112.8	65	1.83	1.71	23.92	53.12
DH59	1	84.13	84.13	59.75	1.82	1.7	21.6	46.65
DH60	1	97.2	97.2	63.5	1.9	1.75	20.07	51.04
DH61	1	121.88	121.88	77.25	1.82	1.62	19.89	52.37

DH62	1	124.73	124.73	73	1.93	1.83	26.61	60.7
DH63	1	100.85	100.85	73.25	1.79	1.83	23.02	56.69
DH64	1	103.85	103.85	68.75	1.9	1.76	21.38	52.44
DH65	2	65.8	65.8	34	1.3	1.38	25.28	43.85
DH66	2	33.53	33.53	29	1.48	1.54	30.63	36.88
DH67	1	82.03	82.03	73	1.86	1.87	25.24	57.37
DH68	2	74.4	74.4	35	1.4	1.52	26.03	37.41
DH69	1	110.1	110.1	86.25	1.98	1.87	26.17	54.15
DH70	1	87.23	87.23	66.75	1.9	1.77	19.93	55.39
DH71	2	82.8	82.8	56.5	1.39	1.44	22.03	32.3
DH72	1	117.85	117.85	70.25	1.83	1.78	19.1	54.11
DH73	2	87.4	87.4	44	1.4	1.42	17.48	43.15
DH74	1	133.5	133.5	82	1.82	1.78	18.29	50.77
DH75	1	135.35	135.35	103	1.98	1.88	17.04	54
DH76	1	87.25	87.25	69.5	2.08	1.85	29.77	53.82
DH77	1	94	94	75	1.8	1.79	22.67	56.37
DH78	1	117.2	117.2	67.33	1.7	1.87	22.79	54.88
DH79	2	72.7	72.7	38.5	1.42	1.53	21.68	46.49
DH81	1	80.1	80.1	78	1.95	1.81	20.75	55.81
DH82	2	35.75	35.75	24	1.46	1.44	21.45	38.48
DH83	1	110.8	110.8	78	1.82	1.87	22.06	53.08
DH84	1	142.2	142.2	80.67	1.78	1.74	22.47	54.98
DH85	2	27.4	27.4	22	1.53	1.46	23.61	46.02
DH86	2	37.4	37.4	36.5	1.54	1.48	19.47	42.93
DH87	2	54.55	54.55	34	1.39	1.41	19.96	37.82
DH88	1	98.43	98.43	68.67	1.77	1.7	19.95	52.05
DH89	1	73	73	73.67	2.02	1.78	22.31	56.02
DH90	2	56.93	56.93	39.25	1.38	1.36	21.3	35.85
DH91	2	79.63	79.63	45	1.47	1.48	19.4	36.5
DH92	2	77.8	77.8	38.33	1.37	1.44	21.92	39.02
DH93	1	116.13	116.13	66.5	1.88	1.71	19.83	51.91
DH94	2	33.2	33.2	26	1.33	1.39	15.39	45.61

DH95	1	84.8	84.8	66.75	1.96	1.75	18.89	58.2
DH96	1	104.93	104.93	57.25	1.8	1.71	21.3	57.76
DH97	1	79.15	79.15	55	1.88	1.69	20.27	53.76
DH98	1	78.1	78.1	69.75	2.03	1.89	23.49	56.92
DH99	1	99.93	99.93	66	1.81	1.77	23.72	53.55
DH100	1	86.73	86.73	46.25	1.85	1.73	20.28	56.05
DH101	1	88.7	88.7	68	1.75	1.69	19.6	50.97
DH102	1	90.43	90.43	62.25	1.75	1.69	21.74	46.69
DH103	1	89.2	89.2	64	1.93	1.74	19.53	54.4
DH104	1	91.47	91.47	67.67	1.89	1.72	22.28	55.83
DH105	1	96.53	96.53	73.25	1.86	1.83	20.6	55.86
DH106	1	112.7	112.7	68.67	1.83	1.77	20.57	56.28
DH107	2	68.2	68.2	24	1.39	1.43	26.32	42.98
DH108	1	93.63	93.63	77.25	2.23	1.94	21.38	54.75
DH109	1	81.7	81.7	68.75	1.97	1.78	20.26	50.71
DH110	1	113.63	113.63	72.67	1.96	1.74	23.7	60.66
DH112	1	86.33	86.33	66.5	1.87	1.88	26.65	57.01
DH113	1	130	130	70.67	1.85	1.85	25.45	52.69
DH115	2	68.1	68.1	35	1.42	1.49	22.05	42.27
DH116	1	131.65	131.65	72.25	1.91	1.72	22.63	55.53
DH117	2	72.2	72.2	33.67	1.46	1.51	25.42	37.04
DH118	2	90.93	90.93	41.33	1.52	1.47	23.49	44.88
DH119	1	96.25	96.25	75.25	2.01	1.89	22.93	56.57
DH120	2	53.93	53.93	39.67	1.35	1.46	20.99	40.88
DH121	2	56.4	56.4	48.5	1.33	1.43	22	37.83
DH122	1	128.58	128.58	70.5	1.9	1.73	21.21	59.67
DH123	1	122.55	122.55	106	1.89	1.66	22.03	51.06
DH124	1	111.97	111.97	72.33	2.02	1.69	26.93	55.62
DH125	2	62.5	62.5	30.75	1.44	1.44	24.75	37.93
DH126	2	40.43	40.43	36.5	1.38	1.43	20.01	48.31
DH127	1	113.13	113.13	68.5	1.9	1.75	21.67	55.14
DH129	1	116.48	116.48	62.25	1.97	1.74	21.51	56.93

DH130	2	54.75	54.75	49.5	1.4	1.44	25.7	21.13
DH131	1	88.33	88.33	72.5	1.86	1.8	22.82	55.15
DH132	1	131.65	131.65	83.5	1.88	1.7	23.51	51.75
DH133	1	86.25	86.25	85.25	2	1.8	24.06	53.12
DH134	2	46.75	46.75	44.5	1.49	1.61	22.81	47.5
DH135	2	60.83	60.83	51.5	1.43	1.5	20.95	45.78
DH136	1	122.78	122.78	89.25	1.85	1.73	23.26	59.68
DH137	1	89.13	89.13	88.25	1.98	1.82	23.1	58.88
DH138	2	79.57	79.57	43	1.4	1.46	21.28	49.22
DH139	2	80.3	80.3	47	1.41	1.5	19.27	47.56
DH140	1	90.33	90.33	75.25	1.94	1.79	23.02	60.35
DH141	1	101.07	101.07	79	1.8	1.68	21.79	54.96

APPENDIX IV: MARKER REGRESSION REPORT

Marker Regression report for set Untitled(Set Name)

Using quantitative trait **Plant Height** _cm

Chi Square Stats, P = 0.05

Additive regression model

With no control for other QTLs.

116 to 138 informative progeny from Progeny Order Internal

Chr	Locus	Stat	%	P	CI	Add
Chr1A	:: wPt_7951U	4.3	3	0.03738	124	-4.63
Chr1A	:: wPt_1426	4.9	4	0.02675	110	-4.98
Chr1A	:: wPt_4765	6.1	4	0.01341	89	-5.45
Chr1A	:: wPt_8347U	4.2	3	0.03955	127	-4.53
Chr1A	:: wPt_7030	4.5	3	0.03378	120	-4.71
Chr2B	:: wPt_7408	8.6	6	0.00341	64	-6.55
Chr2D	:: wPt_9848	8.6	6	0.00336	64	-6.42
Chr2D	:: wPt_1301	12.0	8	0.00053	46	-7.60
Chr2D	:: wPt_3757	5.7	4	0.01741	96	-5.48
Chr2D	:: wPt_8319	6.8	5	0.00889	79	-5.76
Chr2D	:: wPt_4683U	9.1	6	0.00261	60	-6.92
Chr2D	:: wPt_6343	11.0	8	0.00090	50	-7.34
Chr3A	:: wPt_9808	7.5	5	0.00614	73	-6.27
Chr3B	:: wPt_7614	5.3	4	0.02147	102	-5.33
Chr3B	:: wPt_2559	5.4	4	0.01973	99	-5.23
Chr3D	:: WMS456	75.9	42	0.00000	9	-17.48
Chr4A	:: wPt_7280	6.1	4	0.01347	89	5.46
Chr4A	:: wPt_9833	6.6	5	0.00992	82	5.67
Chr4A	:: wPt_9196U	7.3	5	0.00684	74	5.96
Chr4A	:: wPt_4645U	6.7	5	0.00943	81	5.75
Chr4A	:: wPt_5749	6.6	5	0.00992	82	5.67
Chr4A	:: wPt_0105	6.6	5	0.00992	82	5.67

Chr4A	:: wPt_6214	6.9	5	0.00869	79	5.81
Chr4A	:: wPt_4596	7.4	5	0.00657	74	6.02
Chr4A	:: wPt_8000	7.0	5	0.00835	78	5.81
Chr4A	:: wPt_1362	5.6	4	0.01774	96	5.24
Chr4A	:: wPt_2151	4.1	3	0.04327	132	4.54
Chr4A	:: wPt_2084	5.5	4	0.01878	98	5.18
Chr4A	:: wPt_7807	6.3	4	0.01223	86	5.54
Chr4A	:: wPt_2983	5.3	4	0.02136	102	5.34
Chr4A	:: wPt_3795	4.6	3	0.03208	117	4.80
Chr4B	:: wPt_3917	4.0	3	0.04625	135	-4.42
Chr4B	:: wPt_8292	4.9	4	0.02657	110	-4.89
Chr4B	:: wPt_5265	5.1	4	0.02334	105	-5.00
Chr4B	:: wPt_0391	6.2	4	0.01304	88	-5.45
Chr4B	:: wPt_6869	4.8	3	0.02851	112	-4.81
Chr4D	:: wPt_0472	19.0	14	0.00001	30	9.87
Chr4D	:: wPt_0431	5.2	4	0.02278	104	5.13
Chr4D	:: wPt_2379U	3.9	3	0.04940	139	4.32
Chr4D	:: wPt_5809	5.4	4	0.01964	99	5.41
Chr5B	:: wPt_0033	6.4	5	0.01130	85	5.78
Chr6A	:: wPt_7204	3.9	3	0.04721	137	-4.48
Chr6A	:: wPt_8124	5.4	4	0.01958	99	-5.16
Chr6A	:: wPt_9474	5.4	4	0.02032	100	-5.15
Chr6B	:: wPt_4924	4.8	3	0.02910	113	-4.80
6D	:: wPt_8509U	4.3	3	0.03753	125	-4.75
6D	:: wPt_1695	5.7	4	0.01734	96	-5.24
Chr7D	:: wPt_6433U	5.5	4	0.01895	98	-5.44
Chr7D	:: wPt_9660U	4.1	3	0.04202	130	-4.50
Chr7D	:: wPt_6878U	4.0	3	0.04489	134	-4.44

Marker Regression report for set Untitled(Set Name)

Using quantitative trait **Head Height** _mm

Chi Square Stats, P = 0.05

Additive regression model

With no control for other QTLs.

116 to 138 informative progeny from Progeny Order Internal

Chr	Locus	Stat	%	P	CI	Add
Chr2B	:: wPt_7408	5.6	4	0.01796	97	-3.88
Chr2D	:: wPt_9848	6.3	4	0.01216	86	-4.02
Chr2D	:: wPt_1301	9.5	7	0.00203	58	-4.96
Chr2D	:: wPt_8319	4.3	3	0.03864	126	-3.33
Chr2D	:: wPt_4683U	7.4	5	0.00645	73	-4.58
Chr2D	:: wPt_6343	8.5	6	0.00361	64	-4.71
Chr3A	:: wPt_1096U	4.8	4	0.02818	112	-3.83
Chr3A	:: dddU	5.6	4	0.01750	96	-3.99
Chr3A	:: wPt_3887U	4.6	3	0.03211	117	-3.73
Chr3A	:: wPt_9808	5.9	4	0.01520	92	-4.07
Chr3D	:: WMS456	127.1	60	0.00000	6	-15.21
Chr4A	:: wPt_1362	4.0	3	0.04625	135	3.22
Chr4A	:: wPt_2084	3.9	3	0.04969	140	3.16
Chr4A	:: wPt_7807	4.5	3	0.03475	121	3.41
Chr5B	:: wPt_8604	4.7	3	0.03057	115	3.52
Chr5B	:: wPt_1420	4.8	3	0.02887	113	3.49
Chr5B	:: wPt_9666	5.6	4	0.01825	97	3.75
Chr5B	:: wPt_0033	11.0	8	0.00094	50	5.46
Chr5D	:: wPt_1261	7.2	5	0.00744	76	4.25
Chr6A	:: wPt_7204	6.4	5	0.01160	85	-4.14
Chr6A	:: wPt_8124	10.5	7	0.00122	53	-5.17
Chr6A	:: wPt_5696U	5.9	4	0.01537	92	-3.85
Chr6A	:: wPt_4445	5.4	4	0.01996	100	-3.77
Chr6A	:: wPt_9474	5.2	4	0.02225	103	-3.70
Chr6B	:: wPt_2991	3.9	3	0.04769	137	-3.23

6D :: wPt_8509U 4.7 4 0.02957 114 -3.62

6D :: wPt_1695 9.6 7 0.00198 57 -4.93

Chr7A :: wPt_0433 4.5 3 0.03353 119 -3.59

Chr7D :: wPt_9660U 3.8 3 0.04999 140 -3.17

Marker Regression report for set Untitled(Set Name)

Using quantitative trait **Awn Length** _mm

Chi Square Stats, P = 0.05

Additive regression model

With no control for other QTLs.

116 to 138 informative progeny from Progeny Order Internal

Chr	Locus	Stat	%	P	CI	Add
Chr1A	:: wPt_7951U	4.7	3	0.02994	114	-4.90
Chr1A	:: wPt_1426	5.3	4	0.02114	102	-5.25
Chr1A	:: wPt_4765	6.5	5	0.01084	84	-5.69
Chr1A	:: wPt_8347U	4.6	3	0.03185	117	-4.78
Chr1A	:: wPt_7030	4.8	3	0.02783	111	-4.94
Chr2B	:: wPt_7408	10.1	7	0.00147	54	-7.19
Chr2D	:: wPt_9848	9.3	7	0.00224	59	-6.78
Chr2D	:: wPt_1301	12.8	9	0.00034	43	-7.96
Chr2D	:: wPt_3757	6.0	4	0.01449	91	-5.71
Chr2D	:: wPt_8319	7.6	5	0.00599	72	-6.12
Chr2D	:: wPt_4683U	10.0	7	0.00157	55	-7.35
Chr2D	:: wPt_6343	11.9	8	0.00056	46	-7.72
Chr3A	:: dddU	4.2	3	0.04029	128	-4.80
Chr3A	:: wPt_9808	8.0	6	0.00463	68	-6.57
Chr3B	:: wPt_7614	5.7	4	0.01654	94	-5.63
Chr3B	:: wPt_2559	5.9	4	0.01557	93	-5.50
Chr3D	:: WMS456	79.4	44	0.00000	9	-18.02
Chr4A	:: wPt_7280	5.3	4	0.02167	102	5.15
Chr4A	:: wPt_9833	5.2	4	0.02296	104	5.08

Chr4A	:: wPt_9196U	6.3	4	0.01241	87	5.60
Chr4A	:: wPt_4645U	5.2	4	0.02241	104	5.15
Chr4A	:: wPt_5749	5.2	4	0.02296	104	5.08
Chr4A	:: wPt_0105	5.2	4	0.02296	104	5.08
Chr4A	:: wPt_6214	5.4	4	0.02054	101	5.21
Chr4A	:: wPt_4596	5.7	4	0.01654	94	5.40
Chr4A	:: wPt_8000	5.4	4	0.02064	101	5.19
Chr4A	:: wPt_1362	4.7	3	0.03039	115	4.86
Chr4A	:: wPt_2084	4.6	3	0.03220	117	4.79
Chr4A	:: wPt_7807	5.2	4	0.02196	103	5.14
Chr4A	:: wPt_2983	4.3	3	0.03729	124	4.91
Chr4B	:: wPt_3917	4.5	3	0.03485	121	-4.74
Chr4B	:: wPt_8292	5.4	4	0.01957	99	-5.22
Chr4B	:: wPt_5265	5.7	4	0.01713	95	-5.33
Chr4B	:: wPt_0391	6.8	5	0.00931	80	-5.78
Chr4B	:: wPt_6869	5.3	4	0.02161	102	-5.11
Chr4D	:: wPt_0472	17.2	13	0.00003	33	9.56
Chr4D	:: wPt_0431	4.4	3	0.03595	122	4.80
Chr4D	:: wPt_5809	4.5	3	0.03334	119	5.01
Chr5B	:: wPt_0033	6.8	5	0.00933	80	6.01
Chr6A	:: wPt_7204	4.4	3	0.03611	123	-4.80
Chr6A	:: wPt_8124	5.9	4	0.01553	92	-5.42
Chr6A	:: wPt_9474	6.0	4	0.01425	90	-5.51
Chr6B	:: wPt_4924	5.3	4	0.02136	102	-5.13
6D	:: wPt_8509U	5.0	4	0.02508	108	-5.20
6D	:: wPt_1695	6.1	4	0.01364	89	-5.50
Chr7A	:: wPt_0433	3.9	3	0.04867	138	-4.64
Chr7A	:: wPt_6768	4.0	3	0.04460	133	-4.52
Chr7D	:: wPt_6433U	5.9	4	0.01479	91	-5.72
Chr7D	:: wPt_9660U	4.7	3	0.02948	114	-4.88
Chr7D	:: wPt_6878U	4.6	3	0.03158	117	-4.82

Marker Regression report for set Untitled(Set Name)

Using quantitative trait **1000 Seed Weight**

Chi Square Stats, P = 0.001

Additive regression model

With no control for other QTLs.

116 to 138 informative progeny from Progeny Order Internal

Chr	Locus	Stat	%	P	CI	Add
Chr2B	:: wPt_7408	14.6	10	0.00014	38	-2.11
Chr2D	:: wPt_9848	11.5	8	0.00070	48	-1.85
Chr2D	:: wPt_1301	12.2	8	0.00047	45	-1.92
Chr2D	:: wPt_8319	10.9	8	0.00094	50	-1.81
Chr2D	:: wPt_4683U	12.7	9	0.00037	44	-2.03
Chr2D	:: wPt_6343	12.6	9	0.00038	44	-1.96
Chr2D	:: wPt_4413	12.8	10	0.00035	44	-2.15
Chr3D	:: WMS456	27.1	18	0.00000	22	-2.84

Marker Regression report for set Untitled(Set Name)

Using quantitative trait **Grain Shape**

Chi Square Stats, P = 0.05

Additive regression model

With no control for other QTLs.

116 to 138 informative progeny from Progeny Order Internal

Chr	Locus	Stat	%	P	CI	Add
Chr2B	:: wPt_7408	3.9	3	0.04971	140	0.08
Chr2D	:: wPt_1301	6.0	4	0.01469	91	0.10
Chr2D	:: wPt_4683U	6.1	4	0.01368	89	0.11
Chr2D	:: wPt_6343	6.1	4	0.01331	88	0.10
Chr3A	:: dddU	5.7	4	0.01673	95	0.10
Chr3A	:: wPt_9808	8.3	6	0.00395	66	0.12

Chr3D	:: WMS456	330.4	91	0.00000	4	0.48
Chr4A	:: wPt_7280	4.3	3	0.03761	125	-0.09
Chr4A	:: wPt_9833	4.8	3	0.02874	113	-0.09
Chr4A	:: wPt_4645U	4.3	3	0.03855	126	-0.09
Chr4A	:: wPt_5749	4.8	3	0.02874	113	-0.09
Chr4A	:: wPt_0105	4.8	3	0.02874	113	-0.09
Chr4A	:: wPt_6214	4.9	3	0.02754	111	-0.09
Chr4A	:: wPt_1362	4.0	3	0.04577	135	-0.08
Chr4A	:: wPt_7807	4.2	3	0.04046	128	-0.08
Chr4B	:: wPt_0391	4.9	3	0.02732	111	0.09
Chr5B	:: wPt_0033	7.2	5	0.00731	76	-0.11
Chr6A	:: wPt_7204	6.8	5	0.00916	80	0.11
Chr6A	:: wPt_8124	9.0	6	0.00264	61	0.12
Chr6A	:: wPt_5696U	4.4	3	0.03690	124	0.09
Chr6A	:: wPt_4445	4.6	3	0.03174	117	0.09
Chr6A	:: wPt_9474	6.9	5	0.00864	79	0.11
6D	:: wPt_8509U	7.0	5	0.00815	78	0.11
6D	:: wPt_1695	8.3	6	0.00402	66	0.12
Chr7A	:: wPt_0205	5.1	4	0.02397	106	0.10
Chr7D	:: wPt_6460U	7.3	5	0.00672	74	0.12

Marker Regression report for set Untitled(Set Name)

Using quantitative trait **Grain L/W**

Chi Square Stats, P = 0.05

Additive regression model

With no control for other QTLs.

116 to 138 informative progeny from Progeny Order Internal

Chr	Locus	Stat	%	P	CI	Add
Chr2B	:: wPt_4559	4.1	3	0.04295	131	-0.04
Chr3A	:: wPt_1096U	4.8	4	0.02835	112	-0.05
Chr3A	:: dddU	4.2	3	0.03934	127	-0.05

Chr3A	:: wPt_9808	4.9	3	0.02670	110	-0.05
Chr3D	:: WMS456	209.5	78	0.00000	5	-0.23
Chr4A	:: wPt_7524	4.9	3	0.02748	111	-0.05
Chr4A	:: wPt_9833	4.5	3	0.03375	120	0.05
Chr4A	:: wPt_4645U	4.5	3	0.03474	121	0.05
Chr4A	:: wPt_5749	4.5	3	0.03375	120	0.05
Chr4A	:: wPt_0105	4.5	3	0.03375	120	0.05
Chr4A	:: wPt_6214	4.9	4	0.02622	109	0.05
Chr4A	:: wPt_8000	3.9	3	0.04778	137	0.04
Chr4A	:: wPt_1362	5.4	4	0.02049	101	0.05
Chr4A	:: wPt_2151	4.1	3	0.04209	130	0.05
Chr4A	:: wPt_2084	5.0	4	0.02517	108	0.05
Chr4A	:: wPt_7807	5.7	4	0.01653	94	0.05
Chr4A	:: wPt_3795	5.0	4	0.02466	107	0.05
Chr4B	:: wPt_0391	6.0	4	0.01430	90	-0.05
Chr5B	:: wPt_1420	4.0	3	0.04461	133	0.04
Chr5B	:: wPt_9666	4.3	3	0.03872	126	0.04
Chr5B	:: wPt_2586	4.5	3	0.03340	119	0.05
Chr5B	:: wPt_2041	4.3	3	0.03911	126	0.05
Chr5B	:: wPt_0033	12.4	9	0.00043	45	0.08
Chr5D	:: wPt_1261	5.1	4	0.02364	106	0.05
Chr6A	:: wPt_7204	6.2	4	0.01291	88	-0.06
Chr6A	:: wPt_8124	7.9	6	0.00498	69	-0.06
Chr6A	:: wPt_4445	4.4	3	0.03659	123	-0.05
Chr6A	:: wPt_9474	3.9	3	0.04758	137	-0.04
6D	:: wPt_8509U	4.3	3	0.03918	127	-0.05
6D	:: wPt_1695	7.1	5	0.00752	76	-0.06
Chr7D	:: wPt_6460U	6.2	4	0.01286	88	-0.06

Marker Regression report for set (Set Name)

Using quantitative trait **Grain length**

Chi Square Stats, P = 0.001

Additive regression model

With no control for other QTLs.
115 to 138 informative progeny from Progeny Order Internal

Chr	Locus	Stat	%	P	CI	Add
Chr3D	:: WMS456	233.3	82	0.00000	5	-0.76

Marker Regression report for set (Set Name)
Using quantitative trait **Grain width**
Chi Square Stats, P = 0.001
Additive regression model
With no control for other QTLs.
115 to 138 informative progeny from Progeny Order Internal

Chr	Locus	Stat	%	P	CI	Add
Chr2D	:: wPt_8319	12.5	9	0.00040	44	-0.05
Chr5B	:: wPt_5851	14.6	10	0.00013	38	-0.06
Chr5B	:: wPt_4936	12.1	8	0.00050	46	-0.05
Chr5B	:: wPt_4628	10.9	8	0.00095	50	-0.05

Marker Regression report for set marker regression report_grain width(Set Name)
Using quantitative trait **Grain depth**
Chi Square Stats, P = 0.001
Additive regression model
With no control for other QTLs.
115 to 138 informative progeny from Progeny Order Internal

Chr	Locus	Stat	%	P	CI	Add
Chr3D	:: WMS456	73.8	41	0.00000	9	0.14
Chr5B	:: wPt_0033	13.4	9	0.00025	42	-0.07

Marker Regression report for set Untitled(Set Name)

Using quantitative trait **Embryo L/W**

Chi Square Stats, P = 0.05

Additive regression model

With no control for other QTLs.

116 to 138 informative progeny from Progeny Order Internal

Chr	Locus	Stat	%	P	CI	Add
Chr3B	:: wPt_5704	5.5	4	0.01850	97	-0.03
Chr3D	:: WMS456	130.2	61	0.00000	6	-0.13
Chr4B	:: wPt_8292	6.7	5	0.00979	81	-0.04
Chr4B	:: wPt_5265	6.7	5	0.00964	81	-0.04
Chr4B	:: wPt_0391	4.9	4	0.02632	109	-0.03
Chr4D	:: wPt_0472	3.9	3	0.04890	139	-0.03
Chr5B	:: wPt_1420	4.0	3	0.04659	136	0.03
Chr5B	:: wPt_9666	4.0	3	0.04641	136	0.03
Chr5B	:: wPt_0033	9.8	7	0.00173	56	0.04
Chr5D	:: wPt_1261	5.9	4	0.01521	92	0.03
Chr6A	:: wPt_7204	4.0	3	0.04630	135	-0.03
Chr6A	:: wPt_8124	5.0	4	0.02504	107	-0.03
Chr6A	:: wPt_4445	4.5	3	0.03392	120	-0.03
6D	:: wPt_1695	4.5	3	0.03315	119	-0.03
Chr7D	:: wPt_6460U	3.9	3	0.04890	139	-0.03

Marker Regression report for set (Set Name)

Using quantitative trait **Inner crease**

Chi Square Stats, P = 0.05

Additive regression model

With no control for other QTLs.

116 to 138 informative progeny from Progeny Order Internal

Chr	Locus	Stat	%	P	CI	Add

Chr3A	:: wPt_9808	6.1	4	0.01335	89	-1.70
Chr3D	:: WMS456	125.4	60	0.00000	6	-6.23
Chr4B	:: wPt_0391	4.1	3	0.04188	130	-1.32
Chr6A	:: wPt_7204	4.8	3	0.02853	112	-1.48
Chr6A	:: wPt_8124	4.6	3	0.03177	117	-1.43
Chr6A	:: wPt_9474	5.7	4	0.01699	95	-1.59
6D	:: wPt_8509U	5.9	5	0.01516	92	-1.66
6D	:: wPt_1695	4.8	3	0.02840	112	-1.45
Chr7A	:: wPt_7947	5.7	4	0.01698	95	-1.58
Chr7A	:: wPt_2501	6.2	4	0.01279	87	-1.64
Chr7D	:: wPt_7763	4.5	3	0.03337	119	-1.42
Chr7D	:: wPt_6460U	9.1	6	0.00256	60	-2.05

Marker Regression report for set Untitled(Set Name)

Using quantitative trait Out_Crease

Chi Square Stats, P = 0.05

Additive regression model

With no control for other QTLs.

116 to 138 informative progeny from Progeny Order Internal

Chr	Locus	Stat	%	P	CI	Add
Chr1A	:: wPt_7951U	8.4	6	0.00380	65	-0.68
Chr1A	:: wPt_1426	6.7	5	0.00957	81	-0.62
Chr1A	:: wPt_4765	6.1	4	0.01370	89	-0.58
Chr1A	:: wPt_8347U	7.5	5	0.00616	73	-0.64
Chr1A	:: wPt_7030	6.5	5	0.01074	83	-0.60
Chr1A	:: wPt_9757	6.4	4	0.01173	85	-0.59
Chr1B	:: wPt_1403	4.5	3	0.03438	120	-0.50
Chr1D	:: wPt_6560	4.2	3	0.04135	129	-0.49
Chr1D	:: wPt_7437	4.5	3	0.03356	119	-0.52
Chr1D	:: wPt_8545	4.0	3	0.04591	135	-0.49

Chr1D :: wPt_4988 4.0 3 0.04640 136 -0.48
Chr1D :: kkkU 4.0 3 0.04488 134 -0.50
Chr2B :: wPt_7817U 4.0 3 0.04669 136 -0.48
Chr2D :: wPt_1554 4.4 3 0.03581 122 0.51
Chr2D :: wPt_4691U 6.5 5 0.01096 84 0.63
Chr2D :: wPt_6847U 7.2 5 0.00714 75 0.66
Chr2D :: wPt_4144 5.8 4 0.01565 93 0.58
Chr2D :: wPt_4413 6.5 5 0.01098 84 0.63
Chr3A :: wPt_1096U 5.3 4 0.02093 102 -0.57
Chr3A :: wPt_3887U 4.2 3 0.04161 130 -0.52
Chr3A :: wPt_8054U 9.1 7 0.00254 60 0.70
Chr4B :: wPt_6869 4.1 3 0.04189 130 -0.48
Chr5A :: wPt_0605 7.3 5 0.00693 75 0.64
Chr5A :: wPt_7769 5.6 4 0.01770 96 0.56
Chr5A :: wPt_1954 9.7 7 0.00179 56 0.74
Chr5B :: wPt_5851 5.4 4 0.02011 100 0.54
Chr5B :: wPt_4418 5.5 4 0.01946 99 0.55
Chr5B :: wPt_4936 4.2 3 0.03954 127 0.49
Chr5B :: wPt_7101 5.6 4 0.01771 96 0.57
Chr5B :: wPt_2041 4.3 3 0.03762 125 0.49
Chr5B :: wPt_0033 4.7 3 0.02962 114 0.53
Chr5B :: wPt_2305 5.1 4 0.02392 106 -0.53
Chr5B :: wPt_4628 5.4 4 0.01977 100 0.56
Chr5B :: wPt_7413 7.2 5 0.00748 76 0.65
Chr6A :: wPt_3873 4.3 3 0.03889 126 -0.49
Chr6B :: wPt_4924 5.2 4 0.02297 104 -0.53
Chr7B :: wPt_7720 6.3 4 0.01239 87 0.61
Chr7B :: wPt_8938U 4.9 3 0.02740 111 0.53
Chr7B :: wPt_4300 4.0 3 0.04502 134 0.49
Chr7B :: wPt_2356 4.4 3 0.03631 123 0.49
Chr7B :: wPt_6156 5.4 4 0.01992 100 0.56
Chr7B :: wPt_3833 4.6 3 0.03173 117 -0.50
Chr7B :: wPt_6372 4.6 3 0.03118 116 -0.50
Chr7B :: wPt_8561 5.5 4 0.01911 98 0.58

Chr7B	:: wPt_5377U	9.7	7	0.00183	57	0.76
Chr7B	:: wPt_2838U	6.9	5	0.00845	78	0.63
Chr7B	:: wPt_1209	7.4	5	0.00656	74	0.64
Chr7B	:: wPt_5816	5.9	4	0.01497	91	0.58
Chr7B	:: wPt_6673U	6.8	5	0.00901	80	0.61
Chr7B	:: wPt_0504	6.8	5	0.00901	80	0.61
Chr7B	:: wPt_4140	6.8	5	0.00901	80	0.61
Chr7B	:: wPt_7108	7.7	5	0.00555	71	0.65
Chr7B	:: wPt_1196	7.7	5	0.00555	71	0.65
Chr7B	:: wPt_9746	7.6	5	0.00600	72	0.65
Chr7B	:: wPt_2933U	7.2	5	0.00715	75	0.64
Chr7B	:: wPt_9992U	4.8	3	0.02879	113	0.54
Chr7B	:: wPt_9767U	4.1	3	0.04214	130	0.51
Chr7D	:: wPt_6606	9.4	7	0.00216	58	-0.75
Chr7D	:: wPt_8089U	5.1	4	0.02385	106	-0.56
Chr7D	:: wPt_1347U	4.5	3	0.03341	119	-0.52